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B VITAMIN CONTENT IN THE SKELETAL MUSCLE OF THE HORSE FED A B VITAMIN-LOW DIET

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IN A previous paper by Carroll, Goss and Howell (1949) the results of two experiments were reported. The first one showed that horses were able to synthesize seven members of the B Complex vitamins. Thiamin was one of the vitamins synthesized. It was observed in the second experiment that horses suffered from a lack of thiamin when fed a ration of low B-Vitamin content for 20 weeks. One of the two horses used in the second experiment died and the remaining one, a Percheron filly, recovered when her ration was supplemented with thiamin. When this filly had reached her original weight the experiment was terminated. It is conceivable that after a time, she might have suffered from a lack of riboflavin, pantotheinc acid, or some other B Vitamin. To obtain further information on this possibility, it was decided to slaughter the animal and compare the B Vitamin content in the skeletal muscle with that found in horses receiving a normal diet. A decrease of any B vitamin in the skeletal muscle, it was believed, might be an indication of the eventual results from a low intake of this vitamin. It is not assumed in this report that skeletal muscle is a principal storage space for any or all the members of the B Complex, since several workers have observed that other body tissues contain larger amounts.

It is well to mention that Pike (1940), Hughes (1941), Miller *et al.* (1943), and Ellis and Madsen (1944) observed that the thiamin content of the skeletal muscle of pork varied with the amount of thiamin in the ration. Heineman *et al.* (1946) reported similar results and noted that pigs on a low thiamin intake grew less rapidly and utilized feed less efficiently than those on a normal diet.

Experimental

The care and treatment of the Percheron filly used in this experiment are described in the earlier paper by Carroll *et al.* (1949). Her ration was kept low in B vitamin for 32 weeks. During the last 12 weeks, however, thiamin was added. By the end of this period, the original weight of 1325 pounds had been regained. For the purposes of the study reported in this paper, the filly was then slaughtered.

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Sampling

A representative sample was taken from the caecal and the rectal contents. (The rectal contents represented feces.) Another sample (approximately 100 gm.) was cut from the gluteus medius muscle in the hind quarter. These were mixed with equal volumes of 95 percent ethyl alcohol to make final concentrations of about 50 percent alcohol. The pH was adjusted between 4.0 and 6.0, and the samples were stored at a temperature below 0° C. Fresh gluteus medius muscle samples from 12 different horses were obtained at a horse packing plant—these horses being assumed to have had normal diets. Preparation of the samples was identical with that described above. Finally, all samples were dried at room temperature, finely ground in a Wiley Mill, and stored in closed jars at room temperature. The gluteus medius muscle taken from the 12 horses was composited into one sample. This muscle is referred to as skeletal muscle in table 1.

Vitamin Assays

Amounts of the B vitamins were determined by the methods described in the previous paper by Carroll *et al.* (1949). Inositol, however, was assayed according to the method of Beadle (1945).

TABLE 1. VITAMIN CONTENT—MICROGRAMS PER GRAM DRIED MATERIAL

Vitamin	Ration #2*	From filly on ration #2 plus thiamin			From 12 horses on normal feed
		Caecal contents	Rectal contents	Skeletal muscle	Skeletal muscle
Thiamin	<.2	**	**	2.3	1.6
Riboflavin	<.4	6.6	9.3	3.5	7.5
Pantothenic acid	<.2	<.1	8.0	1.8	22.
Nicotonic acid	<.2	51.	15.6	94.0	363.
Pyridoxine	<.01	2.7	10.1	9.8	10.4
Biotin	<.01	.03	0.12	.005	.18
Folic Acid	<.01	5.3	1.4	.16	.57
Inositol	368.	415.	425.	872.	952.

* Ration #2: Plain beet pulp 58%, starch 15%, glucose 15%, washed casein 5%, washed fish meal 5%, spent bone black 2%, salt (NaCl) ad libitum, Vitamin D—10,000 I.U. weekly; and Vitamin A —900,000 I.U. weekly. An average of 30 mg. of thiamin hydrochloride was fed daily during the last 12 weeks of the experiment.

** Since the filly was receiving thiamin, these samples were not analyzed for this vitamin.

Table 1 summarizes the data obtained. Riboflavin, nicotinic acid, pyridoxine, and folic acid were present in higher amounts in the caecum contents than in the ration, thus indicating synthesis of these vitamins. Little or no synthesis of pantothenic acid and biotin was noted in the caecum. From

the amount found in the rectal contents, it was apparent that synthesis of these two vitamins did occur, however, in the alimentary tract somewhere between the caecum and the rectum. The data are inconclusive regarding the synthesis of inositol.

The amounts of riboflavin, pantothenic acid, nicotinic acid, biotin, and folic acid found in the skeletal muscle of the horses receiving normal feed were considerably larger than those in the muscle of the filly receiving the B vitamin-low diet plus thiamin. Apparently, however, sufficient absorption of synthesized pyridoxine occurred to maintain a level almost normal.

Since the inositol in the ration was adequate, no evidence of skeletal muscle depletion of this vitamin was noted. The washing procedure employed to lower the B vitamin content of the ration failed to wash out the inositol. Thirty mg. of thiamin hydrochloride administered daily—approximately 2.5 mg. per 100 pounds body weight—was sufficient to maintain the skeletal muscle at a normal level.

Summary and Conclusion

A Percheron filly, weighting 1325 pounds, was used in this experiment. She had been on a B vitamin-low diet for 32 weeks, the last 12 weeks of which she received an average daily supplement of 30 mg. of thiamin hydrochloride. This filly was slaughtered and the B vitamin content of the gluteus medius muscle was compared with a composite gluteus medius muscle sample obtained from 12 horses receiving normal feed. In addition, samples from the caecal and rectal contents of the filly were analyzed to determine whether B vitamins were being synthesized. The conclusions are:

1. Synthesis of riboflavin, pantothenic acid, nicotinic acid, pyridoxine, biotin, and folic acid was observed in the alimentary tract of the filly receiving a B vitamin-low diet plus thiamin. The data did not indicate synthesis of inositol.

2. The gluteus medius muscle of the filly contained noticeably less riboflavin, pantothenic acid, nicotinic acid, biotin, and folic acid than the composite sample. Though these five vitamins were being synthesized, their absorption was insufficient to maintain the muscle level at that found in horses on normal feed. There was no evidence of inadequate absorption of pyridoxine and inositol.

3. An average daily intake of 30 mg. of thiamin hydrochloride—about 2.5 mg. per 100 pounds body weight—was sufficient to prevent depletion of this vitamin in the skeletal muscle.

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THE EFFECTS OF EGG YOLK ON THE VARIOUS PHYSICAL AND CHEMICAL FACTORS DETRIMENTAL TO SPERMATOZOAN VIABILITY

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THE practice of artificial insemination in the breeding of farm animals has presented many new problems to the investigator. Most of these problems are closely related and are a part of the general problem of maintaining the viability and fertilizing capacity of the spermatozoa for long periods at low termperatures during the storage or transportation of semen.

From the time the semen is collected from the male until the insemination of the female, the spermatozoa are subjected to many types of adverse environmental conditions. Rapid cooling of the semen to the storage temperature subjects the spermatozoa to a temperature shock (Easley, Mayer and Bogart, 1942; Lasley and Bogart, 1943, 1944a, 1944b; Lasley and Mayer, 1944) which kills a varying proportion of the cells. Even at the low temperatures at which semen is generally stored or shipped, harmful metabolic products accumulate at a relatively rapid rate and eventually reach a concentration sufficiently great to kill many of the spermatozoa. The diluters used for increasing the volume of semen introduce other factors detrimental to spermatozoan viability.

Phillips and Lardy (1940) recommended the use of egg yolk in the diluters added to bull semen prior to storage or shipping. Lasley and Mayer (1944) showed that egg yolk provides a factor which enables the spermatozoa to withstand some of the adverse conditions encountered during storage, especially temperature shock. These authors also showed the existence of a variation in the inherent ability of bull spermatozoa to withstand adverse conditions. Mayer and Lasley (1945) designated the ability of spermatozoa to withstand adverse conditions in the absence of egg yolk or the egg yolk resistance factor as "inherent resistance"; whereas the term "acquired resistance" was given to the resistance acquired by an additional number of spermatozoa in a semen specimen in the presence of egg yolk or the egg yolk resistance factor.

The ideal cells for the study and assay of the resistance factor would possess little or no inherent resistance, but would be capable of acquiring resistance in the presence of the resistance factor. Preliminary studies in

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this laboratory indicated that the percentage of spermatozoa in a specimen of stallion semen possessing inherent resistance is small, and that the majority of the spermatozoa in stallion semen can acquire resistance in the presence of egg yolk or other substances containing the resistance factor. The resistance properties of stallion spermatozoa make them ideal cells for the study and assay of the resistance factor of egg yolk. It is the purpose of this paper to report studies of egg yolk-containing diluters and of spermatozoan resistance utilizing stallion spermatozoa as assay cells.

Materials and Methods

Semen was collected once each week from the stallion by use of the Missouri U.S.D.A. artificial vagina (Lambert and McKenzie, 1940). Following collection, the semen was taken to the laboratory where the various evaluation techniques were applied within one half hour from the time of collection. The stallions from which the semen specimens were obtained were highly fertile as indicated by routine laboratory fertility tests and shown by the high settling percentage resulting from services.

The percentages of live spermatozoa after a "cold shock" and following storage periods of 24 and 48 hours were determined by employing the staining method described by Lasley, Easley and McKenzie (1942) and later modified by Easley, Mayer and Bogart (1942).

The "cold shock" was made by subjecting 0.5 cc. of the spermatozoan suspension in a small vial to a temperature of 0° C. for 10 minutes, after which the percentage of live spermatozoa was determined by the staining technique previously described.

In the storage tests, the spermatozoan suspensions in test tubes were placed in a one liter beaker containing 500 cc. of water at a temperature of approximately 20° C. The beaker containing the tubes of semen was then placed in the refrigerator, maintained at 6–8° C., and stored for 24 or 48 hours.

Motility was designated as 0 or by a number from 1 to 5 inclusive, the 5 denoting maximum motility. Since motility rating is influenced by such factors as temperature and pH, precautions were taken to prevent these factors from influencing the results (Bogart and Mayer, 1946).

Results

Resistance of Stallion Spermatozoa

Stallion spermatozoa in undiluted semen have little inherent resistance as measured by the cold shock technique and as indicated by the absence of motility after a storage period of 24 hours and by the failure of stallion spermatozoa to survive a storage period of 48 hours (table 1). However,

when 1 part of semen is diluted with 4 parts of a diluter containing egg yolk, there is a marked increase in the percentage of resistant spermatozoa. Best results were obtained with a diluter composed of equal parts of egg yolk and 5 percent glucose solution. Of interest, are the results obtained with egg yolk in distilled water (table 1).

TABLE 1. THE EFFECT OF EGG YOLK DILUTERS UPON THE ABILITY OF STALLION SPERMATOZOA TO RESIST A COLD SHOCK AND SURVIVE DURING STORAGE

Kind of diluter	Number of determinations	Avg. motility rating after 24 hr. storage	Average percentage of live spermatozoa		
			After 24 hr. storage	After 48 hr. storage	After cold shock(0° C. for 10 min.)**
Egg Yolk 1 part* Water 1 part	6	1.4	73.5	55.0	74.7
Egg Yolk 1 part* 5% glucose 1 part	16	2.9	86.6	81.0	84.5
Egg Yolk 1 part* Phosphate buffer 1 part	21	1.0	80.2	65.9	70.7
Egg Yolk 1 part* Phosphate buffer 1 part 5% glucose 2 parts	9	1.6	71.5	34.5	65.5
Control (undiluted)	19	0.0	25.7	1.7	43.8

* In all experiments the rate of dilution was 1 part semen to 4 parts of diluter.
** Made prior to the storage period immediately following dilution.

The Role of Glucose in the Storage of Stallion Semen

Evidence has been presented (Mayer and Lasley, 1945) that the egg yolk resistance factor is soluble in an aqueous solution. Mayer and Lasley (1945) have also shown that the quantity present in egg yolk is sufficient to enable one to obta n a positive demonstration of the factor at dilutions as high as 1 part of egg yolk to 200 parts of diluter. It is evident then, that the superior results obtained by the glucose-yolk diluter (table 1) are not due to a greater solubility of the factor in glucose solution than in the other diluters used.

Since the egg yolk-glucose diluter was superior to all others, particularly during storage, it appeared that the glucose might have properties similar to the resistance factor.

When a solution of glucose is used in a diluter, either with or without a buffer, the survival during storage is greater but the number surviving a

cold shock is not greatly increased. This would preclude resistance effects of the glucose solution.

Glucose differs from other substances used for the preparation of diluters in this investigation in that it is non-ionizable in solution and is utilizable by spermatozoa for energy production. Either of these characteristics may be responsible for its superiority as a constituent of a diluter for the storage of spermatozoa. However, as shown in table 2, sucrose as a diluter constituent

TABLE 2. THE RESISTANCE AND STORAGE SURVIVAL OF STALLION SPERMATOZOA DILUTED WITH SOLUTIONS CONTAINING GLUCOSE AND SUCROSE

Kind of diluter	Number of samples	Average percentage of live spermatozoa		
		After 24 hrs. storage	After 48 hrs. storage	After cold shock (0° C. for 10 min.)*
5% aqueous glucose	12	51.8	32.7	49.0
5% glucose 1 part Phosphate buffer 1 part	6	38.7	10.3	49.0
10% aqueous sucrose	2	74.0	56.0	69.0
10% sucrose 1 part Egg yolk 1 part	1	85.0	85.0	91.0
5% glucose 1 part Egg yolk 1 part	16	86.0	81.0	85.0
Control (undiluted)	19	25.7	1.7	43.8

* Made immediately following dilution prior to storage.

gave results during storage which equalled those obtained with glucose. Moore and Mayer (1949) demonstrated the absence of enzymes in stallion and other mammalian spermatozoa necessary for the utilization of sucrose. On the basis of the results obtained with sucrose, it would seem that the superior results obtained with glucose are not necessarily due to its utilization in glycolytic processes. That glucose does not possess any resistance or protective properties is demonstrated in table 2 by the results of the cold shock technique and by the failure of glucose to protect stallion spermatozoa from the harmful effects of bile salts which, in the absence of egg yolk, kill spermatozoa in a short time. On the other hand, the results following storage (table 2) clearly indicate a beneficial effect of glucose and of sucrose. Perhaps the beneficial effects both of glucose and of sucrose may be attributed to

their lack of ionization in solution and to the absence of certain ions. These observations were the basis of the assumption that certain ions in high concentration may be harmful to mammalian spermatozoa.

Diluters Containing Ionized Salts and Spermatozoan Survival

In this laboratory M/8 phosphate buffer with a pH of 7.35 has been used as the diluter for most semen studies, while in some laboratories M/15 phosphate buffer is used. Other laboratories recommend the use of a number of different sodium or potassium salts in diluters. It should be noted that Moore

TABLE 3. EFFECT OF DEGREE OF IONIZATION UPON SURVIVAL SPERMATOZOA DURING STORAGE AND THEIR RESISTANCE TO A COLD SHOCK

Kind of diluter	Average percentage of live spermatozoa		
	After 24 hrs. storage	After 48 hrs. storage	After cold shock (0° C. for 10 min.)
Undiluted	45	0	55
5% glucose	76	53	67
Isotonic phosphate buffer	64	29	56
Isotonic citrate solution	74	37	56
Isotonic sodium carbonate	46	0	75
Egg yolk 1; 5% glucose 1	92	92	88
Egg yolk 1; phosphate 2	84	81	60
Egg yolk 1; citrate 2	88	74	56
Egg yolk 1; carbonate 2	86	84	79
Egg yolk 1; sodium chloride 2	44	30	38

and Mayer (1949) find no metabolizable sugar in stallion semen and, consequently, there should be no need for buffering on the acid side during storage since no lactic acid would be formed. Since stallion spermatozoa show little resistance to adverse conditions, investigations were initiated to determine the effects of diluters containing different sodium and potassium salts upon these spermatozoa.

Harmful effect of sodium ions. Apparently certain ions may affect the resistance of stallion spermatozoa as shown by the results presented in table 3. Sodium and potassium phosphate, which possess a relatively high degree of ionization in solution, are more detrimental to survival of stallion spermatozoa during stroage or cold shock than glucose which is not ionizable in solution. In semen diluted with isotonic buffer solutions of the relatively highly ionizable phosphate salts (table 3), the survival of spermatozoa during storage was poor and the percentage survival after a cold shock was compar-

able to that obtained in undiluted semen. Sodium citrate, less highly ionizable than the phosphate salts, was perceptibly superior to the latter in diluters for the storage of stallion spermatozoa (table 3). Sodium carbonate was decidedly inferior to glucose or sodium citrate in increasing the survival of stallion spermatozoa during storage, but both glucose and sodium carbonate afforded some protection in the cold shock. The glucose solution was superior to any of the buffer solutions for the storage of stallion semen (table 3). The spermatozoan survival during storage was apparently inversely related to the degree of ionization of the substances in the diluters.

To test further the effects of ions upon the survival during storage and upon the resistance of stallion spermatozoa, solutions of phosphate, citrate and carbonate buffers alone were compared with solutions of these buffers in combination with egg yolk. When egg yolk was used in combination with these buffer solutions, it practically eliminated their harmful effect; but in no case was a buffer-egg yolk diluter equal to the glucose-egg yolk diluter.

Since a 0.9 percent solution of sodium chloride is extensively used in mammalian tissue studies and is one of the most highly ionized salts, it was used with egg yolk as a diluter. However, in this case, egg yolk was not able to eliminate all the harmful effects of the isotonic sodium chloride solution (table 3). Since, in this laboratory, sodium chloride alone had always given very poor results in the storage of semen from all species of farm animals, it was not compared with the buffers in this experiment. From previous studies one would assume that none of the spermatozoa would have survived the storage of 24 hours.

The effect of substituting potassium for sodium in the phosphate buffer. It is possible by using mono- and di-sodium and mono- and di-potassium phosphates to obtain buffer solutions alike in all respects except for the relative proportions of the sodium and potassium ions. Four buffers, composed of monosodium + disodium phosphate, monosodium + dipotassium phosphate, monopotassium + disodium phosphate, and monopotassium + dipotassium phosphate, were compared alone and in combination with egg yolk. The all sodium phosphate buffer was superior to the combinations of sodium and potassium and to the all potassium phosphate buffers. A diluter composed of a buffer in combination with egg yolk was more satisfactory in every case than a diluter containing the buffer alone.

The effect of varying the anion of buffer salts used in diluters. Buffer solutions composed of sodium phosphates, sodium citrate, or sodium carbonate were used alone and in combination with egg yolk. A diluter containing sodium citrate alone was superior to diluters containing sodium phosphate or sodium carbonate alone for the storage of stallion spermatozoa. The survival after a 48 hour storage period was 37.0 percent in the sodium citrate solution.

Survival in the phosphate buffer was only 9.2 percent, whereas none survived the 48 hour storage period in the sodium bicarbonate buffer. However, the percentage of stallion spermatozoa surviving a cold shock was greater in a diluter containing sodium carbonate alone (75.5%) than in a diluter containing the sodium phosphate (48.3%) or the sodium citrate alone (56.0%). When each of these salts was combined with egg yolk in the diluter a greater number of spermatozoa survived storage and the cold shock than when these salts were used alone. The combination of sodium carbonate and egg yolk in the diluter proved superior to all others both in the percentage survival during storage and in the percentage survival following the cold shock.

Osmotic pressure and pH. Phosphate buffer at pH 6.5, 7.2 and 7.8 were compared alone and in combination with egg yolk. No differences in the effects of the diluters at the various pH levels were observed. It is evident that pH changes between 6.5 and 7.8 have little effect upon stallion spermatozoa during a 48 hour storage period.

In order to ascertain the effect of changes in the osmotic pressure of the diluter upon stallion spermatozoa, diluters containing M/8 phosphate buffer and M/15 phosphate buffer alone and in combination with egg yolk were compared. It has been shown that M/8 phosphate buffer is isotonic with stallion spermatozoa (Easley, Mayer and Bogart, 1942; and Moore and Mayer, 1949). The isotonic (M/8) buffer without egg yolk proved superior to the M/15 phosphate buffer alone as shown by the survival percentages following storage and the cold shock. However, egg yolk protects the spermatozoa from drastic changes in the concentration of diluter constituents.

Discussion

Progress in the investigation of the resistance factor and the physiology of spermatozoan resistance has been limited because no cell had been discovered which possessed the necessary characteristics for the detection and assay of the active factor. Stallion spermatozoa differ from bull, ram, boar, and rabbit spermatozoa in that stallion spermatozoa possess little or no inherent resistance yet surpass all the spermatozoa mentioned in their ability to acquire resistance in the presence of the resistance factor. These characteristics necessary for the detection and assay of the resistance factor in egg yolk adapt stallion spermatozoa for investigations of spermatozoan resistance and of the resistance factor as demonstrated by the results of this investigation.

Although investigators in this laboratory have generally employed the percentage of spermatozoa surviving a cold shock as a measure of resistance,

spermatozoa may manifest resistance to many other adverse environmental conditions. The ability of spermatozoa to survive a long storage period suggests that they may be resistant to changes in pH and osmotic pressure, and to harmful metabolic products. The comparative studies of spermatozoan resistance to various adverse environmental conditions, when suspended in diluters with and without added egg yolk, present further evidence that this type of cellular resistance factor or factors does not protect the spermatozoa solely against rapid or drastic temperature changes. In these experiments, egg yolk enables stallion spermatozoa to survive during storage even though they are subjected to abnormal levels of pH or osmotic pressure, or to the

TABLE 4. THE EFFECT OF VARYING THE OSMOTIC PRESSURE OF THE BUFFER UPON SURVIVAL OF STALLION SPERMATOZOA

Kind of diluter	Average percentage of live spermatozoa		
	After 24 hrs. storage	After 48 hrs. storage	After cold shock (0° C. for 10 min.)
M/8 phosphate buffer	72.0	4.0	60.0
M/8 buffer+egg yolk equal parts	95.0	95.9	79.0
M/15 phosphate buffer	48.0	0.0	43.0
M/15 buffer+egg yolk equal parts	91.0	85.0	88.0

presence of high concentrations of detrimental ions. These results show further that resistance to a cold shock and resistance to other adverse conditions are rather closely associated. Hence, the percentage of the spermatozoa in an egg yolk diluter surviving a cold shock is indicative of the percentage that will survive during storage in the same diluter and an index to the general resistance of these spermatozoa to drastic changes in their environment.

The results of this study indicate that the harmful effects of certain salts upon stallion spermatozoa during storage are related, in some manner, to the degree of ionization of these salts. Further, the comparative results, presented in table 4, are indicative that the anion as well as the cation may be a contributing factor in the poor storage results obtained with certain ionized buffer salts in the absence of egg yolk.

Lasley and Mayer (1944) and Mayer and Lasley (1945) have shown that egg yolk apparently contains some factor or factors which protect spermatozoa from drastic temperature changes and from certain unidentified detrimental conditions encountered during storage at low temperatures. This factor (or factors) can be isolated in a comparatively pure fraction constitut-

ing but a small proportion of the original egg yolk, hence it must be a substance present in the original egg yolk capable of exerting its effects independently. This factor has been designated the spermatozoan resistance factor.

However, on the basis of the results of the present investigation in which whole egg yolk as a part of the diluting medium was able to protect spermatozoa from the detrimental effects of certain anions and cations, from the effects of anisotonic diluters, and from toxic substances (not included in this report), it appears that factors other than this spermatozoan resistance factor are operating. These factors may be either of a physical or chemical nature and may be difficult to duplicate in synthetic storage media.

Conclusions

1. Stallion spermatozoa have little or no inherent resistance to adverse conditions, whereas they are capable of acquiring resistance to these same adverse conditions in the presence of the spermatozoan resistance factor or factors in egg yolk.

2. Since stallion spermatozoa are extremely susceptible to the adverse effects of environmental changes and yet are capable of acquiring resistance to these environmental changes in the presence of the spermatozoan resistance factor, it is evident that these cells are well suited for the detection of the spermatozoan resistance factor and for studies on factors influencing spermatozoan survival.

3. These studies with stallion spermatozoa show that the spermatozoan resistance factor, present in egg yolk (possibly in union with other physical or chemical properties of egg yolk) not only protects spermatozoa from drastic temperature changes; but, in addition, protects them from many types of adverse environmental conditions such as changes in pH and osmotic pressure, and the presence or accumulation of harmful substances.

4. There is an apparent association between the degree of ionization of the salts in a spermatozoan diluter and the harmful effects of these salts upon spermatozoa.

5. Apparently, the favorable results obtained with isotonic glucose and sucrose solutions in this investigation using stallion spermatozoa may be attributed to the low degree of ionization of these substances in solution rather than to any role they may play in spermatozoan metabolism.

6. On the basis of the results of this investigation, it is recommended that the combination of egg yolk with an isotonic solution either of sucrose or of glucose be used in the dilution of stallion semen for optimum survival of spermatozoa during storage.

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SODIUM, POTASSIUM, AND CHLORINE CONTENT OF FEEDING STUFFS

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THERE is a paucity of data in the literature on the sodium and chlorine content of feeding stuffs. This was noted when sodium and chlorine low rations were being designed from the information contained in the summaries of Black *et al.* (1939), Beeson (1941), and Morrison (1948). Such rations were desired for their use in the determination of sodium and chlorine requirements of growing pigs. Preliminary experiments, Meyer *et al.* (1949), with growing pigs showed that a protein supplement of linseed meal and wheat flour middlings when fed with corn did not produce a sodium chloride deficiency while the use of soybean oil meal as a protein supplement to corn caused a salt deficiency to become apparent. These results were unexpected in view of the sodium values given in the literature which indicated that soybean oil meal contained approximately four times as much sodium as linseed meal and that the chlorine content of the two feeds was the same. The recent development of the flame photometer provided a new method for the determination of sodium and potassium which is both accurate and rapid. Hence a survey of the sodium, potassium, and chlorine content of feeding stuffs was undertaken to expedite future research involving sodium, chlorine, and potassium.

Experimental

The sodium and potassium analyses were made with the Perkin-Elmer model 52A flame photometer. This model is equipped with an internal standard circuit which adds to the accuracy of analysis. A description of a similar model is reported by Berry *et al.* (1946). The feed samples were prepared by wet ashing with nitric and perchloric acids as described by Gieseking *et al.* (1935). Wet ashing was used because less equipment and less time was required in ashing; and because, as shown by St. John and Midgley (1941, 1942) and Cholak and Hubbard (1944), less loss by volatilization and manipulation occurs. In the preliminary work it was also found that more consistent results were obtained with duplicates and recoveries when the wet ashing method was used. The ash was extracted with 0.055 M nitric

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acid in sufficient volume for use with the standard curves of the flame photometer. The ash was stirred well to dissolve all the soluble salts and allowed to stand for at least half an hour. If necessary, the solutions were filtered to remove the insoluble salts, and analyses were then carried out as prescribed for the instrument.

The chlorine was determined by the procedure of the Association of Official Agricultural Chemists (1945).

Each feeding stuff sample was obtained from a different source, and Na, K, and Cl determinations were made on each sample.

Results and Discussion

The percentages of sodium, potassium, and chlorine in the feeds analyzed are given in table 1 expressed as the percentage of the air dried feed. Moisture determinations were made and the percentage dry matter agrees very closely with the figures given by Morrison (1948) and consequently are not reported. The range in concentration is given when more than one sample of feed was analyzed.

Sodium

The sodium content of the grains and grain by-products was quite constant between samples of the same feed with the exception of corn gluten feed. Apparently the sodium content varies with the manufactured brand of corn gluten feed which may or may not be due to the inclusion of corn solubles. The sodium values, generally, were much lower than the values expressed in the summaries of Black *et al.* (1939), Beeson (1941), and Morrison (1948). On the other hand the values for grains and grain by-products are in close agreement with the human food analyses of Bills *et al.* (1949) obtained with a flame photometer. The lower values obtained with the flame photometer can be explained by the fact that in the classical chemical methods of sodium analysis, the sodium and potassium salts are weighed together after the other metals have been removed by various means. The potassium is removed as chloroplatinate and sodium calculated by difference. According to this method one would expect error especially when applied to feeds very low in sodium. If this is true, the flame photometer would eliminate much of this error and give a closer value of the true content of feeds low in sodium.

The sodium content of the plant protein supplements was lower than the values given by Black *et al.* (1939) and Morrison (1948), but the values were consistent from sample to sample of given feed. Peanut oil meal was an exception in this regard and varied from 0.01 to 0.23 percent sodium. Among the animal protein supplements, fish meal, meat scraps, dried skimmilk,

TABLE 1. SODIUM, POTASSIUM, AND CHLORINE CONTENT OF FEEDS (%)

Feeding stuff	No. samples	Sodium	Potassium	Chlorine
Alfalfa hay, good	5	0.07 (0.01 –0.13)*	1.36 (0.92 –1.72)*	0.19 (0.09–0.44)*
Alfalfa leaf meal, good	6	0.11 (0.07 –0.14)	2.28 (1.72 –2.67)	0.53 (0.49–0.57)
Alfalfa meal, dehydrated	3	0.06 (0.02 –0.14)	2.20 (1.86 –2.68)	0.31 (0.12–0.58)
Barley, common	5	0.01 (0.005–0.02)	0.47 (0.33 –0.54)	0.12 (0.09–0.16)
Bone meal, steamed	6	0.43 (0.37 –0.54)	0.02 (0.008–0.04)	0.03 (0.02–0.06)
Brewers' grains, dried	4	0.02 (0.005–0.04)	0.04 (0.01 –0.08)	0.03 (0.02–0.04)
Buttermilk, dried	5	1.46 (0.45 –2.97)	1.53 (1.21 –1.80)	1.18 (0.85–1.37)
Casein, crude	3	0.06 (0.04 –0.07)	0.08 (0.07 –0.09)	0.26 (0.23–0.28)
Casein, alcohol extracted	5	0.008 (0.001–0.03)	0.01 (0.001–0.06)	0.08 (0.03–0.20)
Clover hay, Ladino	2	0.14 (0.10 –0.17)	1.49 (1.32 –1.65)	0.15 (0.14–0.15)
Corn, dent, Grace No. 2	7	0.004 (0.001–0.009)	0.34 (0.27 –0.45)	0.06 (0.05–0.07)
Corn gluten feed	5	0.21 (0.02 –0.52)	1.06 (0.72 –1.44)	0.37 (0.16–0.64)
Corn gluten meal	5	0.04 (0.02 –0.05)	0.53 (0.37 –0.81)	0.12 (0.08–0.15)
Cottonseed meal	5	0.02 (0.01 –0.04)	1.26 (1.15 –1.35)	0.07 (0.05–0.12)
Distillers'solubles, dried	4	0.26 (0.15 –0.44)	2.10 (1.72 –2.57)	0.43 (0.16–0.68)
Fish meal	6	0.60 (0.30 –0.81)	0.79 (0.57 –0.97)	0.66 (0.43–1.04)
Fish solubles, condensed	4	2.52 (2.03 –3.43)	2.22 (1.61 –2.77)	4.63 (2.83–6.89)
Linseed meal	5	0.05 (0.04 –0.08)	1.11 (0.92 –1.39)	0.04 (0.02–0.06)
Meat scraps	5	0.85 (0.64 –1.07)	0.42 (0.21 –0.55)	0.71 (0.59–0.84)
Oats	5	0.008 (0.005–0.01)	0.51 (0.40 –0.69)	0.10 (0.05–0.19)
Pea seed, field	3	0.006 (0.004–0.01)	0.90 (0.72 –1.01)	0.09 (0.04–0.12)
Peanut oil meal	3	0.12 (0.01 –0.23)	0.82 (0.68 –0.94)	0.21 (0.04–0.37)
Rye grain	3	0.002 (0.001–0.002)	0.41 (0.37 –0.45)	0.06 (0.04–0.07)
Skimmilk, dried	5	0.51 (0.44 –0.56)	1.74 (0.88 –2.37)	1.12 (0.67–1.28)
Soybean oil meal, expeller proc.	6	0.03 (0.006–0.09)	1.98 (1.75 –2.37)	0.07 (0.02–0.13)
Soybean oil meal, solvent proc.	2	0.02 (0.01 –0.02)	1.85 (1.55 –2.15)	0.04 (0.03–0.04)
Tankage, 60% grade	5	1.04 (0.89– 1.16)	0.51 (0.42 –0.56)	1.11 (0.99–1.16)
Timothy hay, good	3	0.008 (0.007–0.01)	1.32 (1.04 –1.64)	0.14 (0.08–0.18)
Wheat	5	0.002 (0.001–0.003)	0.34 (0.18 –0.45)	0.06 (0.05–0.07)
Wheat bran	5	0.006 (0.003–0.008)	1.01 (0.70 –1.29)	0.07 (0.05–0.10)
Wheat flour middlings	3	0.01 (0.006–0.02)	0.78 (0.69 –0.83)	0.06 (0.02–0.09)
Wheat standard middlings	5	0.004 (0.001–0.009)	0.73 (0.34 –0.92)	0.07 (0.05–0.09)
Yeast, brewers', dried	5	0.05 (0.005–0.21)	1.33 (0.60 –2.24)	0.12 (0.04–0.32)
Yeast, irradiated, dried	2	0.41 (0.32 –0.50)	1.05 (0.97 –1.12)	0.41 (0.33–0.49)

* Lowest and highest value.

tankage, and crude casein were remarkably constant in their range of sodium, but dried buttermilk and alcohol extracted casein varied a great deal. The values did agree, however, with the forementioned summaries.

Dried brewers' yeast varied in the amount of sodium to a marked degree depending on the brand of manufacture, and dried distillers' solubles were somewhat similar in this respect. The alfalfa products did not vary a great deal in their content of sodium but the content seemed to depend on the source.

Chlorine

The chlorine content of the feeds was in agreement with the previously mentioned summaries of Black *et al.* (1939), Beeson (1941), and Morrison

(1948). The values for chlorine were quite constant between samples of the same feed with the exception of corn gluten feed, alcohol extracted casein, dried buttermilk, dried distillers' solubles, dried brewers' yeast, and peanut oil meal.

Potassium

The potassium content of the feeds was quite constant with the exception of dried brewers' yeast, and is in agreement with the summaries of Black *et al.* (1939), Beeson (1941), and Morrison (1948). The feeds with a quite low potassium content were brewers' dried grains and bone meal.

Summary

A survey of the sodium, potassium, and chlorine content of the more common feeding stuffs has been made and results are reported.

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CREEP-FEEDING OF NURSING PIGS

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THE purpose of these tests was to study the value of creep-feeding pigs when the sows were fed on pasture as follows: (a) full-fed ear corn twice daily with access to minerals, and (b) self-fed shelled yellow corn and a mixed protein-mineral supplement free-choice. Three series of experiments involving six trials and 134 sows and litters were conducted to determine the value of creep-feeding pigs.

Experimental Procedure and Results

I. *Creep-Feeding of Pigs Has Greatest Value When Sows Are Hand-Fed a Poor Ration*

In experiments 1, 2 and 3, ear corn was full-fed on the ground by hand twice daily and the sows were given free access to a mineral mixture and rye pasture. As suitable sows became available during the spring months, they were placed in groups of three sows and litters on one-fourth acre lots of fall-seeded common rye. The pigs in alternate groups were given access to shelled corn and protein supplement in a self-feeder located in a creep, while the others were forced to eat with the sows. The lactation ration fed in these trials was not as satisfactory as is generally recommended, but was chosen to make the test of the value of the creep as pronounced as possible.

The average results of the three tests on creep-feeding spring pigs on rye pasture are presented in table 1. These data show that more of the creep-fed pigs survived to weaning age. The creep-fed pigs made very significantly ($P<0.01$) larger average daily gains of 0.06 pound or about 12 percent. Twenty percent less feed was required per unit of gain in the creep-fed lots in which 512 pounds of feed was consumed per 100 pounds net gain of sows and pigs compared to 640 pounds for the no-creep lots. The feed consumption of sows and litters was slightly higher in the creep-fed lots due primarily to the extra feed the pigs consumed in the creeps. The average daily creep ration consisted of 73 percent shelled corn and 27 percent supplement—a ration that contains about 21 percent crude protein. This was the creep ration selected by the pigs to supplement sow's milk, rye

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TABLE 1. THE VALUE OF CREEP-FEEDING OF NURSING PIGS ON RYE PASTURE WHEN SOWS ARE HAND-FED ONLY EAR CORN

(Average of Experiments 1, 2 and 3)

Items compared	No creep	Creep[2]
All sows and litters full-fed ear corn twice daily with minerals[1] free-choice		
Av. number of sows	11.7	11.7
Av. number of pigs started	91.0	90.3
Av. number of pigs finished	84.3	88.7
Av. initial litter age (days)	15.7	16.7
Av. days on test	40.3	39.0
Av. initial wt. of sows, lb.	532.3	529.3
Av. gain of sows, lb.	−49.6	−48.0
Av. initial wt. of pigs,[3] lb.	9.0	9.7
Av. gain of pigs,[3] lb.	19.7	21.3
Av. daily gain of pigs,[3] lb.	0.49	0.55**
Av. daily feed for sow and litter, lb.		
Ear corn	17.54	17.09
Shelled corn	—	.72
Supplement	—	.27
Mineral	.22	.20
Total	17.76	18.28
Feed for 100 lb. gain, sow :		
Ear corn (computed as sh. corn)	629.7	470.7
Shelled corn	—	25.0
Supplement	—	9.3
Mineral	10.3	6.7
Total	640.0	511.7

[1] Limestone 2 parts, bone meal 2 parts, salt 1 part.

[2] Shelled corn and supplement (tankage 2 parts expeller soybean meal 1 part) free-choice.

[3] For the pigs that finished.

** P <0.01 highly significant by analysis of variance (Snedecor, 1946).

pasture and the corn that the pigs consumed with the sows. In these tests, the value of the creep was greatest when the season was unfavorable.

II. *Less Advantage for Creep-Feeding Pigs When Sows Are Fed a Good Ration*

The purpose of experiments 4 and 5 was to study the value of a pig-creep for suckling pigs when their dams were self-fed shelled yellow corn and supplement (200 lb. meat and bone scraps, 100 lb. expeller soybean meal, and 5 lb. iodized salt free-choice on pasture.

As suitable sows became available, they were grouped (usually three sows and their litters in each group) on one-fourth acre fall-seeded Balbo rye pastures during the spring and on bromegrass-bluegrass pastures during

the fall of 1948. Twenty-four spring-farrowed and 10 fall-farrowed litters were used. All sows and litters had access to shelled yellow corn and supplement in a self-feeder at all times. Alternate lots of pigs also had access to the following ration in a creep:

Ground yellow corn	45.5%
Broken oat kernels	25.0%
Meat and bone scraps	12.0%
Expeller soybean meal	12.0%
Alfalfa leaf meal	5.0%
Iodized salt	0.5%
Total	100.0%
Crude protein content	20.75%

The data in table 2 indicate that there was no significant advantage in creep-feeding the pigs when the sows and litters had access to a "good

TABLE 2. VALUE OF CREEP-FEEDING PIGS WHEN SOWS ARE SELF-FED SHELLED CORN AND SUPPLEMENT ON PASTURE

(Summary of Experiments 4 and 5)

Items compared	No creep	Creep
All sows and litters self-fed shelled yellow corn and supplement,[1] free-choice		
Number of sows	17	17
Number of pigs started	134	133
Number of pigs finished	130	123
Av. initial pig age, days	12.5	12.4
Av. days on test	43.6	43.1
Av. initial weight of sow, lb.	471.7	486.0
Av. gain, sow, lb.	−30.8	−31.0
Av. initial weight per pig,[2] lb.	7.9	7.5
Av. gain per pig,[2] lb.	21.4	21.2
Av. daily gain per pig,[2] lb.	0.49	0.49
Av. daily feed, sow and litter, lb.		
Shelled yellow corn[3]	12.17	11.40
Supplement	1.73	1.31
Creep ration	—	0.36
Total	13.90	13.07
Feed for 100 lb. gain, sow and pigs, lb.		
Shelled yellow corn	401.4	404.8
Supplement	57.1	46.4
Creep ration	—	12.7
Total	458.5	463.9

[1] Supplement = 200 lb. meat and bone scraps, 100 lb. expeller soybean meal and 5 lb. iodized salt (50.25% crude protein).

[2] For pigs that finished.

[3] Shelled yellow corn contained 8.69% crude protein.

TABLE 3. VALUE OF CREEP-FEEDING PIGS WHEN SOWS ARE SELF-FED ON RYE PASTURE

(Experiment 6)

Items compared	No creep	Creep
All sows and litters self-fed shelled yellow corn and supplement,[1] free-choice		
Number of sows	15	15
Number of pigs started	114	116
Number of pigs finished	104	111
Av. initial pig age, days	12.1	12.2
Av. days on test	43.9	43.9
Av. initial weight of sow, lb.	485.6	452.4
Av. gain per sow, lb.	−34.9	−33.0
Av. initial weight per pig,[2] lb.	7.0	6.8
Av. gain per pig,[2] lb.	23.2	24.3
Av. daily gain per pig,[2] lb.	0.53	0.56
Av. daily feed, sow and litter, lb.		
Shelled yellow corn	11.05	9.84
Supplement	1.36	1.43
Creep ration:		
Shelled yellow corn	—	0.10
Broken oat kernels	—	1.01
Supplement[3]	—	0.22
Total	12.41	12.60
Feed for 100 lb. gain, sow and pigs, lb.		
Shelled yellow corn	383.4	294.2
Supplement	47.3	42.6
Creep ration:		
Shelled yellow corn	—	3.1
Broken oat kernels	—	30.1
Supplement[3]	—	6.7
Total	430.7	376.7

[1] Supplement = 200 lb. meat and bone scraps, 100 lb. expeller soybean meal and 5 lb. iodized salt.

[2] For pigs that finished.

[3] Supplement fed in creep = 100 lb. meat scraps (55% crude protein), 100 lb. solvent soybean meal and 2 lb. iodized salt.

ration"—shelled corn and supplement—self-fed free-choice on rye pasture (1948 spring) or bromegrass-bluegrass pasture (1948 fall).

Although the creeps were conveniently located so that the pigs would use them, the consumption of the creep rations was so small (0.36 lb. per litter daily) that it would not have been expected to exert a significant effect. There was ample self-feeder space for the pigs to eat with the sows without much competition.

III. *Palatability of Feeds in Creep Important*

In other Illinois investigations, Carroll (unpublished data) offered feeds in separate compartments of self-feeders to two different groups of pigs

during the latter part of the suckling period and for a short time after weaning. The pigs ate more shelled corn and broken oat kernels than they did of all other feeds combined. The percentage of protein supplement consumed ranked third in amount. The great preference of pigs for shelled corn over ground corn and the very low consumption of finely ground feeds such as wheat middlings and ground oats was noteworthy.

Since the consumption of the mixed creep ration in experiments 4 and 5 was only 0.36 lb. per litter daily, a comparison of creep versus no-creep was made under similar conditions in experiment 6 except that shelled corn, broken oat kernels and supplement were offered free-choice in the creep. The sows were self-fed the same ration as was fed in experiments 4 and 5 and were on excellent Balbo rye pastures (table 3).

These results show that ten pigs were lost in the no-creep lot while only five pigs were lost in the creep lot. The average daily gain per pig was 0.03 pound greater in the creep lot, which was not a statistically significant difference ($P = 0.10$). The average daily creep ration (1.33 pounds) consisted of about 8 percent shelled yellow corn, 76 percent broken oat kernels and 16 percent supplement, and contained about 21 percent crude protein. Although the average daily feed per sow and litter did not differ greatly, the feed required per 100 pounds of gain of sows and pigs was 377 pounds in the creep lot and 431 pounds in the no-creep lot, or 12.5 percent less feed was required per unit of gain in the creep lots.

Summary

The value of the pig creep for nursing pigs was studied in six experiments with 134 sows and their litters on pasture.

An average of the first three experiments showed that spring pigs creep-fed a simple ration of shelled yellow corn and supplement (2 tankage, 1 soybean meal) made 12 percent faster daily gains and suffered fewer death losses than those not creep-fed when sows were fed a "poor ration" of ear corn (full-fed by hand twice daily) and minerals on rye pasture. The sows and litters in the creep-fed lot required 20 percent less feed per unit of gain than those in the no-creep lot.

In two comparisons (experiments 4 and 5) there was no significant advantage in creep-feeding pigs a mixed ration when the sows and litters were self-fed shelled yellow corn and supplement free-choice on rye pasture (spring) or brome-bluegrass pasture (fall). The daily consumption of the mixed creep-ration was so small (0.36 lb. per litter) that it would not have been expected to exert a significant effect.

In the sixth experiment, the sows and pigs were self-fed shelled yellow corn and supplement free-choice on Balbo rye pasture with alternate lots of pigs being offered shelled corn, broken oat kernels and supplement free-

choice in a creep. During the test 9 percent of the pigs died in the no-creep lot while only 4 percent of the pigs died in the creep lot. A slight, but statistically insignificant, increase in gains was obtained by creep-feeding. The average creep-ration consisted of about 8 percent shelled yellow corn, 76 percent broken oat kernels and 16 percent supplement, and contained 21 percent crude protein. In the creep lot, 12.5 percent less feed was required per 100 pounds of net gain of sows and pigs.

Conclusions

Within the limits of the conditions imposed in these experiments, the following conclusions seem justified:

1. When sows and litters are full-fed ear corn by hand, the creep-fed pigs gain very significantly more rapidly than the no-creep pigs. The sows and litters in the creep-fed lot require about 20 percent less feed per 100 pounds of gain, and fewer pigs die than in the no-creep lot.

2. Less advantage for creep-feeding exists if self-feeding an adequate ration to sows is practiced.

3. Feed consumption from the creep was greater when palatable feeds were offered free-choice than when a complete mixed ration was shelf-fed, indicating the desirability of free-choice feeding such feeds as shelled corn, broken oat kernels and supplement. Palatability of the creep ration is important.

4. Since creep-feeding was quite beneficial and economical in most cases and in no cases was it detrimental, creep-feeding of nursing pigs is recommended under most conditions, particularly under the following: (a) when sows are hand-fed in groups; (b) when large numbers of sows and litters are running together, increasing hazards to the pigs; (c) when sows are fed limited rations which would prevent the pigs from having access to an adequate supply of supplementary feeds; and (d) when sows are self-fed balanced rations and most uniform rapid and economical performance of the pigs is desired.

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ADFQUACY OF SYNTHETIC DIETS FOR GROWTH AND REPRODUCTION OF SWINE[1]

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HOGAN and Anderson (1948) reviewed the earlier literature on the nutritional requirements of swine, and reported that pigs receiving a synthetic diet grew at a normal rate, though they did not attain a maximum rate of growth. There has been some interest in the vitamin content of the antipernicious anemia fraction of liver and Cartwright, Wintrobe and Humphreys (1946) reported that a pig developed normocytic anemia on a synthetic diet that contained casein (Labco[2]) and sulfasuxidine, and was cured by intramuscular injections of refined liver extract. Cartwright, Fay, Tatting and Wintrobe (1948) carried out a similar experiment but included both sulfasuxidine and a folic acid antagonist in the diet. The animals became anemic and one responded slightly to administration of the APA liver extract. The relief was complete in all animals on administration of folic acid and their behavior would indicate a severe deficiency of folic acid and a mild deficiency of the APA factor.

Neumann, Krider and Johnson (1948) gave pigs a synthetic diet and obtained practically the same rate of growth as did Hogan and Anderson (1948). However, when the pigs were given intramuscular injections of the APA liver extract the gain from the 3rd to the 8th week was almost twice the gain made on the basal diet alone.

The observations of Nichol, Dietrich, Cravens and Elvehjem (1949) on chicks, and of Johnson and Neumann (1949) on pigs indicate that vitamin B_{12} is the active agent in APA liver extracts. Neumann, James, Krider and Johnson (1949) gave pigs a synthetic diet which contained isolated soybean protein as a source of nitrogen and found that supplementation with vitamin B_{12} produced the maximum growth response and improved hematopoiesis. Symptoms of vitamin B_{12} deficiency were irritability, sluggish movements, and weakness in the hind legs.

After Hogan and Anderson (1948) had demonstrated that young pigs grow at a satisfactory rate on a synthetic diet it was decided to investigate the possibility that the growth rate would be raised higher still by adding the antipernicious anemia liver fraction to the basal diet. The results obtained with this material are described in the following pages.

[1] Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 1199.
[2] The Borden Company.

Experimental

The experimental procedure was described by McRoberts and Hogan (1944). The pigs were allowed to nurse the sow until they were two days old since in our earlier experience the mortality rate had been high, even on diets believed to be adequate, if the pigs failed to receive colostrum. The experimental rations described in table 1 were of the synthetic type, homogenized with water during the first 8 weeks to give a solids content of 19 percent. After that time the ration was supplied in dry form. Since it is essential to keep young pigs warm, they were quartered in a room held at a temperature of 75° F. or higher.

TABLE 1. COMPOSITION OF EXPERIMENTAL RATIONS

Ingredient	Ration No.			
	330	336	337	340
	%	%	%	%
Casein (Labco)	15	30	30	15
Sucrose	70	—	30	69.5
Cerelose (dextrose)	—	30	—	—
Cornstarch	—	5	5	—
Lard	5	30	30	5
Salt Mixture[1]	5	5	5	5
Cellulose (woodpulp)	5	—	—	5
Vitamin B_{12} Conc.[2]				0.5

Vitamins[3] per 100 gm., all rations					
Vitamin A	I.U.	2000	Pyridoxine HCl	mg.	1
Vitamin D	I.U.	400	Ca-Panthothenate	mg.	3
Vitamin E	mg.	1	Nicotinic Acid	mg.	4
Vitamin K_3	mg.	2	Choline-Cl	mg.	100
Thiamine HCl	mg.	1	Inositol	mg.	100
Riboflavin	mg.	1	Biotin	mg.	0.03
			Pteroylglutamic Acid		0.2

[1] Richardson and Hogan (1946).

[2] APF Supplement No. 3, Merck & Co.

[3] Vitamins A and D were purchased as a concentrate from Mead Johnson and Company, Evansville, Indiana. The pteroylglutamic acid was generously supplied by Dr. T. H. Jukes, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. All other vitamins were generously supplied by Dr. D. F. Green of Merck and Company, Inc., Rahway, New Jersey.

Ration 327 contained 4 mg. of vitamin E but was otherwise identical with Ration 337.

Ration 334 did not contain folic acid but was otherwise identical with Ration 327.

Two separate trials were conducted to determine whether antipernicious anemia concentrates contain a factor that is important in swine nutrition. The growth rates of the four pigs in the first trial up to 8 weeks of age, and their rations, were described in a previous publication (Hogan and Anderson, 1948). Two pigs, 60 and 61, received Ration 327 while pigs 56 and 64

received Ration 334. When the pigs were 9 weeks of age the two lightest were selected for daily intramuscular injections of antipernicous anemia liver extract.[3] An acceleration of the growth rate was observed on the third day after the injections were started and, as shown in table 2, the two lightest pigs had become the heaviest by the time they were 15 weeks old. At this time Pigs 60 and 64 were discarded. No. 61 was changed to Ration 334 and during the remainder of the experimental period Nos. 56 and 61 consumed the same ration. Additional evidence of the effect of the APA factor is shown in table 4, which contains the data recorded during the reproductive stage of No. 56.

TABLE 2. EFFECT OF THE ANTIPERNICIOUS ANEMIA FACTOR ON THE GROWTH RATE OF PIGS. TRIAL 1

Ration No.	327		334	
Pig No.	60	61	64	56
Age, days	Kg.	Kg.	Kg.	Kg.
63	17.3	15.7 210 U.S.P. Units[1]	13.4 210 U.S.P. Units[2]	18.1
77	21.8	23.4	21.3	23.8
91	27.4	31.8	30.6	31.0
105	33.8	42.4	38.6	35.8

[1] The antipernicious anemia liver fraction was administered from the 63rd to the 91st day, in 28 doses of 7.5 units each.

[2] The liver fraction was administered from the 63rd to the 77th day, in 14 doses of 15 units each.

The preliminary trial just described indicated that the addition of APA to the purified rations increased the rate of growth and another trial was carried out in an attempt to confirm this observation. The experimental rations were homogenized in water as usual but they were supplied in open containers to which the pigs had access at all times and they were taught to drink on the first day. The pigs were Chester Whites, 2 days old, divided into 4 groups of 2 pigs each. The experimental procedure and the gains in weight by the pigs are summarized in table 3.

The effect of the antipernicious anemia liver fraction[4] on the rate of growth was tested by administering 18.75 U.S.P. units to each pig in Group C by intramuscular injection, beginning when they were 5 days of age. During the first 2 weeks after the first injection the response was slight. In the following 4 or 5 weeks the rate of gain was accelerated and the appearance of the pigs improved. At the end of 8 weeks the pigs that received the

[3] Eli Lilly and Company, Indianapolis, Indiana. According to Register, Lewis, Thompson and Elvehjem (1949) this preparation contains approximately 1 mcg. vitamin B_{12} per U.S.P. unit.

[4] Courtesy of Dr. R. A. Brown, Parke, Davis & Company, Detroit, Michigan

injections had an average weight of 12.1 Kg., while the controls in Group B on Ration 336 weighed 8.1 Kg. During the 8th week the pigs in Group C did not gain more rapidly than did their controls, and in the following week each pig received a total of 37.5 U.S.P. units of the liver fraction by injection. There was an immediate response and when the pigs were 93 days old the injected group had an average weight of 25.0 Kg. The controls, in Group B, had an average weight of 15.6 Kg.

TABLE 3. THE EFFECT OF THE ANTIPERNICIOUS ANEMIA FACTOR ON THE GROWTH RATE OF PIGS. TRIAL 2

Ration No.	A 337		B 336		C 336		Cow's milk	
No. of pig	1F	6M	3F	5M	4F	1M	3M	7M
Age, days	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.
0	1.4	1.3	1.2	1.2	1.0	1.2	1.3	1.3
5	2.1	1.8	1.7	2.1	1.7 (18.75)[1]	1.9 (18.75)	2.0	1.9
23	4.7	3.7	3.7	3.5	4.0	5.0	6.4	7.0
56	14.6	10.4	9.1	7.2	11.8	13.3	25.0	24.2
63	14.9	10.5	10.4	8.8	12.6 (37.5)	15.2 (37.5)		
70	15.0	8.9	12.2	9.8	16.1	17.9		
93	25.5	14.2 (60.0)	17.0	14.2 (60.0)	26.6 (60.0)	24.4		
101	28.5	16.7	18.0	15.5	29.7	28.3		
119	38.4	24.2	19.6	21.8	38.5	37.9		
133		30.9	22.6	27.0				
140		34.8						

[1] The figures in parentheses are U.S.P. units of the antipernicious anemia factor injected during the indicated age interval.

As a further test of the nutritional value of the antipernicious anemia factor this material was supplied to one pig in each of the groups A, B and C, beginning on the 93rd and ending on the 101st day. The pigs selected to receive the supplement were the least thrifty in appearance and in two of the three groups they were the lightest. Each of the 3 pigs received 60 U.S.P. units of the APA factor, divided in 4 equal doses, administered orally. Pig 6M in Group A deserves special mention. It had gained only 3.8 Kg. from 56 to 93 days of age, its hair coat was long, rough and dull, and it had begun to gnaw at the partitions and floors. This pig responded almost immediately to the liver extract therapy and continued to gain satisfactorily until it was 140 days old, when it was discarded. Pig 5M in Group B began to grow more rapidly and it too improved in appearance, but the response was less striking and was of shorter duration. Pig 4F, Group C, continued

to grow well but it is uncertain whether or not the additional supply of the liver extract was required to maintain that rate of growth.

Another point that seems significant is the response of the positive controls, Group C, which received a ration of cow's whole milk, supplemented with 60 gm. sucrose, 2.5 gm. ferrous sulphate, 0.2 gm. cupric sulphate, 0.2

TABLE 4. HISTORY OF SOWS USED IN STUDIES ON ADEQUACY OF SYNTHETIC RATIONS FOR REPRODUCTION

Age	Sow No.			
	Weight	56	Weight	61
days	Kg.		Kg.	
0	1.3		1.6	
2	1.6	Ration 334	1.8	Ration 327
56	16.3		13.4	
63	18.1		15.7	63rd to 91st day 30 U.S.P. units of APA liver fraction
91	30.8		31.3	
105	35.8		42.4	Ration 334
129	40.3	Ration 330	62.5	Ration 330
207	106.1	Loss of appetite, diarrhea	134.7	Feed intake limited to present overweight.
225	114.3		143.8	
230	108.9	230th to 235th day 67.5 U.S.P. units of APA liver fraction.	145.6	
235	110.2		150.6	
249	112.0	Appetite regained, no diarrhea	154.2	
295	140.6	1,000 U.S.P. units gonadotrophic hormone, bred	178.7	
373	179.2	Ration 340[1]	211.4	Ration 340
407	217.7		217.3	
409	206.4	Litter of 7, pigs abnormal, none survived.	217.7	
450	204.1		222.3	
		sows slaughtered for examination		

[1] Ration 340 contained 10 mcg. vitamin B_{12} per lb. of feed.

gm. manganous sulphate, and 0.02 gm. potassium iodide per liter. The pigs that consumed the fortified cow's milk weighed almost twice as much at eight weeks, when they were discarded, as did the pigs in Group C which received injections of the APA factor. It is suggested that cow's milk supplied an important unrecognized vitamin in addition to vitamin B_{12} and other nutrients that may have been present in the liver extract fraction.

Several workers in investigations with other animals have reported that

different carbohydrates have different nutritional properties when incorporated in synthetic diets. Johnson (1949) had found that with his experimental procedure glucose is superior to sucrose as a constituent of synthetic rations for young pigs and we compared these two sugars, as shown in table 3, Groups A and B. Under our experimental conditions the superiority of glucose was not evident. There was some diarrhea in both groups, as reported by Hogan and Anderson (1948), but it did not interfere seriously with the development of the pigs.

Two of the females in the first trial, Nos. 56 and 61, were retained after the others were discarded in an attempt to carry them through a reproductive cycle. Some of the more pertinent data are recorded in table 4. When Gilt 56 was 207 days old she began reducing her feed intake, became listless and had frequent attacks of diarrhea, of variable duration and severity. At the age of 226 days her condition was complicated with pneumonia and it was feared that she would die. In the next five days she was given a total of 67.5 U.S.P. Units of the antipernicious anemia fraction. She recovered quickly and the injections were discontinued. It is believed the improvement was due to vitamin B_{12}, which is supposed to be the active agent in the prevention of pernicious anemia. Gilt 56 was observed in estrus before the attack of pneumonia, but following that time no estrous periods were observed and when she was 295 days old she was given an injection of 1,000 U.S.P. units of pregnant mare serum gonadotropic hormone and bred.

Some time later we were able to obtain a considerable quantity of a concentrate of vitamin B_{12},[5] which is presumably one constituent of the Animal Protein Factor. This concentrate was included in the ration, to make Ration 340, when the gilts were 395 days old just 2 weeks before Sow 56 farrowed. It is believed that this change improved the ration, but if so it was too late to be helpful. There were 7 pigs in the litter with an average weight of 2.2 lbs. All of them had shortened, kinked tails and one had a hare lip. By the end of the 3rd week all of the pigs had died and post mortem examination indicated that starvation and hemorrhagic enteritis were the immediate cause. Sow 56 had little milk in her udder when she farrowed, she never showed a normal desire to nurse her pigs and never secreted enough milk to meet their requirements.

It will be recalled that Sow 38, described in a previous publication (Hogan and Anderson 1948), became anemic while suckling a litter, and died. Sow 56 did not become anemic, and she remained thrifty in appearance although she did not consume large amounts of feed. The performance of Sows 38 and 56 indicates strongly that synthetic diets such as No. 337 are

[5] Courtesy of Dr. D. F. Green, Merck & Co., Rahway, New Jersey.

inadequate for brood sows. It remains to be seen whether they would be adequate if supplemented with liberal amounts of the antipernicious anemia factor.

Sow 61 grew normally and was bred at the age of 226 days. She did not conceive and was bred again 21 days later but again she did not conceive. At the end of the experimental period both gilts were slaughtered for post mortem examination. No abnormalities were found except occlusion of the Fallopian tubes in Sow 61.

Summary

1. When pigs are reared on a synthetic ration the rate of growth is accelerated by the administration of an antipernicious anemia liver fraction.

2. Pigs grow much more rapidly on fortified cow's milk than they do on a synthetic ration even when the antipernicious anemia fraction is administered..

3. Of two sows that had been reared on a synthetic ration, one was unable to conceive. The other bore a litter but most of the pigs were deformed and all were dead by the end of the third week.

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THE ROLE OF MANGANESE IN GROWTH, REPRODUCTION, AND LACTATION OF SWINE[1]

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MANGANESE has not generally been considered a critical element in swine rations. In view of the report of Skinner *et al.* (1928) which indicates that corn and corn by-products are extraordinarily low in manganese, it is entirely possible that a ration indigenous to the corn belt area might be marginal in its manganese content for maximum growth and well-being of swine.

Johnson (1943) reports that pigs made good growth on a ration containing less than ½ ppm of manganese, and that reproduction was satisfactory when the manganese was increased to 6 ppm. Keith and co-workers (1942) found that pigs grew well on low manganese rations, but observed an abnormal gait that could be prevented although not cured with additional manganese.

Materials and Methods

Chester White and Poland China hogs from the station herd were used in all experiments. Except for one feeding trial, where older pigs were used, the pigs were approximately 9 weeks of age when they were placed on growth experiments. Sows used for the reproduction and lactation studies were representative animals selected from each of the lots in 2 of the growth experiments. All animals were confined on concrete and housed in a central type house with outside runways.

The first 4 growth experiments were conducted over a 2-year period (1945–1947) and included spring and fall farrowed pigs. One second generation gilt was carried through growth and reproduction in each of the lots.

The No. 1 basal ration was designed to be low in manganese.

*Gr. yellow corn	75.6
Soybean oil meal	12.0
Gluten meal	6.0
Gr. alfalfa	5.0
Gr. limestone	0.9
Iod. salt	0.5

* Fish press water was substituted for 5% of the corn in 2 experiments where gilts where kept on for reproduction studies.

After completion of 4 growth trials, 2 more basal rations were introduced that contained natural feedstuffs with higher amounts of manganese. All rations were self-fed.

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Basal No. 2

Gr. oats	89.5
Soybean oil meal	2.0
Tankage	2.0
Linseed meal	2.0
Gr. alfalfa	3.0
Gr. limestone	1.0
Iod. salt	0.5

Basal No. 3

Gr. yellow corn	71.5
Tankage	6.0
Lnseed meal	6.0
Gr. alfalfa	15.0
Gr. limestone	1.0
Iod. salt	0.5

Periodic manganese determinations were made on the basal rations according to the method of Willard and Greathouse (1917) modified for colorimetric use.

Levels of 40, 80, and 160 ppm of manganese were added to the No. 1 basal ration in the first 4 growth experiments, and 40 ppm was supplemented as indicated to each of the 3 basal rations in the last 2 experiments.

The manganese was added as $MnSO_4 \cdot H_2O$, the source of which was Techmangan.[3]

Representative animals were selected from each lot of one growth experiment for the assay of plasma vitamins A and C; from two experiments to determine bone, ash, and dressing percentage.

Vitamins A and C determinations were made according to the methods described by Kimble (1939) and by Mindlin and Butler (1938) respectively.

Blood, liver, kidney, and pancreas tissue samples were taken from animals from each lot of some of the growth experiments for manganese analysis.

Criteria used to measure reproduction and lactation performance were fertility, number of pigs farrowed, weight of pigs at birth, number of pigs raised, and weight at weaning.

Results and Discussion

Table 1 shows the summary of 4 growth experiments, where manganese was added at levels of 40, 80, and 160 ppm to basal No. 1. Periodic Mn analysis of the basal ration revealed a Mn content of approximately 12 ppm. The pattern of results was consistent for each of the 4 experiments. In every case the pigs in lot 2 made the greatest daily gain and the most efficient use

[3] A technical grade of feeding manganese provided through the courtesy of Tennessee-Eastman Corp., Kingsport, Tenn., through Dr. J. E. Magoffin. It contained 65 percent manganese sulfate.

TABLE 1. THE PERFORMANCE OF GROWING AND FATTENING PIGS ON VARIOUS LEVELS OF MANGANESE

Item considered	Basal No. 1	Basal+ 40 ppm Mm	Basal+ 80 ppm Mn	Basal+ 160 ppm Mn
Total no. pigs on experiment	35	35	35	35
Av. no. days on experiment	123	113.5	121	124
Av. initial wt., lb.	69.2	70.3	69.1	69.9
Av. final wt., lb.	219.5	224.7	220.5	220.3
Av. daily gain, lb.	1.19	1.33*	1.24	1.20
Av. daily feed, lb.	5.15	5.44	5.13	5.21
Feed per cwt. gain, lb.	431.4	408.1	415.0	435.3

* Significant at the 5 percent level as compared with Basal No. 1.

of feed, whereas lots 1 and 4 were low in each of the trials. The daily rate of gain shown by the pigs receiving a supplement of 40 ppm manganese was significantly higher than that of lots 1 and 4. All other gain comparisons did not differ significantly. Although there was an appreciable difference in feed consumption per hundredweight gain between lots 1 and 2 this difference was not significant even at the 5 percent level. It is of interest to note that the performance of pigs in lots 3 and 4 was little or no better than that of those on the basal. If a supplement of 40 ppm Mn had a stimulating effect, why did doubling and quadrupling this amount erase the advantage? One might speculate that a toxic level was reached somewhere in the range of 50 to 100 ppm of Mn. To further check this depressing effect of additional manganese another experiment was conducted, the results of which are discussed later and shown in table 3.

Dressing percentage was determined on a total of 32 animals. Four representative animals were selected from each lot at the close of 2 growth experiments to get slaughter information. The differences were only slight and the variation that was observed was undoubtedly related to the weight of

TABLE 2. MANGANESE CONTENT OF ORGANS FROM HOGS FED VARYING LEVELS OF MANGANESE

Sample	Manganese ppm (dry weight basis)			
	Lot 1	Lot 2	Lot 3	Lot 4
Liver	8.8	9.6	9.7	10.3
Kidney	6.9	7.2	7.4	7.3
Pancreas	5.6	5.3	5.9	5.9

the pigs. The pigs in lots 1 and 4 had the lowest and highest yield respectively, but they also had the lowest and highest average live weight.

Bone ash was determined on 8 pigs from each treatment selected from 2 growth experiments. The third ribs were used for the ash determinations. This information was sought because Keith and co-workers (1942) reported an abnormal gait in pigs on low manganese rations and it was postulated that manganese might have a function in bone formation. However, the bone ash information under the conditions of this experiment gave no indication

TABLE 3. 40 PPM OF MANGANESE AS AFFECTING NATURAL RATIONS LOW, MEDIUM, AND HIGH IN MANGANESE CONTENT

Items considered	Low manganese feeds		High manganese feeds		Medium manganese feeds	
	Basal No. 1	Basal+ 40 ppm Mn	Basal No. 2	Basal+ 40 ppm Mn	Basal No. 3	Basal+ 40 ppm Mn
Total no. pigs on experiment	15	15	15	15	15	15
Av. no. days on experiment	172.5	161.5	176.5	176.5	165.5	165.5
Av. initial wt., lb.	36.2	35.8	36.1	35.0	35.0	35.3
Av. final wt., lb.	221.0	219.8	204.9	209.8	221.8	222.0
Av. daily gain, lb.	1.06	1.12	0.95	0.98	1.14	1.12
Av. daily feed, lb.	4.29	4.31	4.61	4.63	4.69	4.71
Feed per cwt. gain, lb.	405.2	386.4	487.2	471.0	411.9	419.3

of such a relationship. Hogs on the basal ration showed the highest bone ash but was not significantly higher than that of the other lots.

Supplemental manganese had no effect on the blood plasma levels of vitamins A and C. There was more variation between pigs within lots than between lots, and the lot averages were not correlated with the levels of supplementary manganese. The plasma A and C values of all lots fell within the normal range reported by Grummer and co-workers (1948).

The manganese contents in samples of liver, kidney, and pancreas from pigs of lots 1, 2, 3, and 4 are summarized in table 2. The data represent average values for samples from 4 pigs in each lot for 2 experiments. A portion of each organ analyzed was dried and the manganese determined on an ashed aliquot by the periodate method. Although the rate of storage was not proportional to the level of manganese fed, there was a tendency for manganese to concentrate in the livers from pigs fed rations with added manganese. The differences in levels of manganese in the kidney and pancreas were even less pronounced than those shown by the liver.

Results of the growth experiments reported in table 1, where the low manganese basal was used, suggested the design of another experiment. This experiment was instigated to find what effect 40 ppm of manganese would have when added to natural rations containing greater amounts of manganese. Basal rations 2 and 3 contained 33 and 18 ppm of manganese respectively. Basal No. 1 was checked along with the 2 new rations and each served as a control. Table 3 is a summary of 2 growth experiments. Forty ppm of manganese again stimulated rate of gain when added to basal No. 1, although not significantly but failed to improve the growth performance of pigs fed basals Nos. 2 and 3. This suggests that the critical level

TABLE 4. MANGANESE SUPPLEMENTATION AS RELATED TO REPRODUCTIVE AND LACTATING PERFORMANCE OF SOWS

Lot no.	Treatment	No. of sows	No. sterile	Av. no. of heat periods per conception	Av. no. of pigs farrowed per conceived sow	Av. no. of pigs weaned	Av. wt. per pig weaned
							lb.
1	Basal No. 1	12	3	1.8	5.7	3.0	19.3
2	Basal+ 40 ppm Mn	14	2	1.2	7.1	4.6	20.2
3	Basal+ 80 ppm Mn	13	3	1.4	8.6	2.6	16.7
4	Basal+160 ppm Mn	11	1	1.2	6.7	4.8	17.4

of manganese required by growing pigs lies somewhere between that amount of manganese found in basal No. 1 and basal No. 3 (12–20 ppm). However, a lesser supplemental amount than 40 ppm might have stimulated growth of the pigs fed the basal No. 2 and No. 3 rations. It is also possible that the presence of certain minerals in rations Nos. 2 and 3, not found in basal No. 1, inhibited stimulation by the supplemental manganese.

If a slight toxic effect was encountered when the total manganese is above 50 ppm of the ration then basal No. 3 and No. 4 would not be expected to be improved by an addition of 40 ppm of manganese.

To further investigate possible harmful effects of high levels of manganese, 500 ppm of manganese were added to the basal No. 1 ration. The supplemental lot (8 pigs) averaged .97 pound daily gain and required 453 pounds of feed per cwt. gain as compared to 1.21 pound gain and 428 pounds feed for the unsupplemented group. Five hundred ppm of manganese definitely retarded appetite and growth especially during the latter part of the trial. Pigs in the treated group exhibited a stiffness of limbs and stilted gait toward the end of the experiment.

There were no significant differences between treatment groups (table 4)

when reproduction and lactation performance was compared, although the results indicate a tendency for the basal-fed sows to be less fertile on the basis of number of sterile sows, heat periods required for conception, and number of pigs farrowed. Gross examinations of the reproductive tracts of sterile sows showed congenital obstructions to fertilization that could not be associated with the plane of nutrition. The number of pigs weaned and weaning weights were extremely poor for all lots which undoubtedly was a reflection of the nutritional inadequacy of the basal ration. The sows showed evidence of poor lactation and the pigs scoured frequently during the suckling period. One sow, fed the unsupplemented ration, farrowed a litter with deformed front legs. All other pigs appeared to be normal at birth.

Summary

A ration of corn, soybean oil meal, gluten meal, and alfalfa, containing about 12 ppm of Mn, was improved for pigs confined on concrete by supplementing additional manganese. However, the performance was not improved when the total manganese content exceeded 55 ppm. Growing hogs did not tolerate a high level of manganese (500 ppm). The specific manganese requirement of growing pigs is still an unknown quantity. Costly synthetic diets will perhaps be necessary for such a determination.

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CHOLINE VERSUS BETAINE AND EXPELLER VERSUS SOLVENT SOYBEAN MEAL FOR WEANLING PIGS[1]

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RESEARCH at the Illinois Station by Dyer *et al.* (1949) showed that a ration composed of corn and expeller soybean meal and fortified with minerals vitamins A and D and 5 vitamins of the B complex (thiamine, riboflavin, niacin, pantothenic acid, and pyridoxine) was significantly improved for weanling pigs by the addition of either choline or methionine. However Ferrin (1946) stated that the rate of gain was depressed when choline was added to a similar corn-soybean meal ration that contained dried whey and alfalfa meal. Schaefer *et al.* (1949) reported that vitamin B_{12} has a sparing effect on the choline requirement of the chick and rat.

It is known that the dispensable choline can be replaced by certain other methylating agents. McKittrick (1947) reported that choline, above the indispensable level, may be replaced by betaine in the chick diet. Mishler *et al.* (1949) stated that betaine had a higher supplementary value than choline or methionine when added to a simplified corn-soybean meal ration for chicks.

Few experiments have been conducted in which solvent soybean meal has been compared with expeller soybean meal as protein supplements for weanling pigs in drylot. Since more solvent soybean meal is being produced, more data as to its comparative value with expeller soybean meal are needed. Robison (1941) reported that toasted solvent soybean meal fed as the only protein supplement or in combination with other protein supplements produced a slightly faster rate of gain than did expeller soybean meal fed to pigs under the same conditions. Hostetler and Boling (1936) reported that solvent and expeller soybean meals produced about the same average daily gains. Bethke and Sweet (1939) found that toasted solvent soybean meal promoted as rapid growth as expeller soybean meal in chicks.

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[2] This study was conducted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Animal Science.

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Other investigators have reported that expeller and solvent soybean meals produced almost equal gains in heavier pigs.

The objects of this study with weanling pigs fed in drylot were: (1) to study the interchangeability of choline and betaine as methyl group donors (the amount of methyl groups from the two sources being equated) when added to a corn-soybean meal ration; (2) to compare solvent extracted soybean meal with expeller soybean meal as protein supplements; and (3) to compare a "special" solvent soybean meal with expeller and solvent soybean meal as protein supplements. The three soybean meals were fed in rations equated to contain 20% crude protein.

Experimental Procedure

Six comparable lots of nine 56 day-old Duroc pigs each were group fed to final weights of about 90 pounds in drylot. Feed and water were offered *ad libitum*. The basal ration consisting of corn and soybean meal was fortified with minerals, vitamins A and D and 6 vitamins of the B-complex (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine and B_{12} concentrate) and supplied 20% crude protein. Blended expeller soybean meal was used as the protein supplement in the first 3 lots. Pigs in lots 1, 2 and 3 were fed identical rations except that 25 gm. choline chloride and 21 gm. betaine were added per 100 pounds of feed in lots 2 and 3 respectively. The rations used

TABLE 1. COMPOSITION OF BASAL RATIONS FED

Ingredients	Ration 1 Expeller soybean meal	Ration 4 Solvent soybean meal	Ration 6 Special solvent soybean meal
Ground yellow corn	63.5	64.5	64.5
Expeller soybean meal (blended) (42%)[1]	34.0	—	—
Solvent soybean meal (44%)[2]	—	33.0	—
Special solvent soybean meal (44%)[3]	—	—	33.0
Vitamin A & D oil (2000 I.U. vitamin A, 800 I.U. vitamin D per gram)	2.0	2.0	2.0
Mineral mixture (H & K)[4]	0.5	0.5	0.5
B-vitamin additions[5]	+	+	+

[1] A blend of equal parts of 42% protein expeller soybean meal from three sources was used.

[2] Received 60 minutes of heat treatment in which time a maximum temperature of 230° F. was reached.

[3] A minimum temperature of 212° F. was maintained for 30 minutes in presence of steam at atmospheric pressure.

[4] Composition of H & K Mineral mixture (% or lbs.)

Iodized salt	25	Potassium carbonate	.20
Steamed bonemeal	31	Copper sulfate	.10
Ground limestone	40	Manganese sulfate	.56
Magnesium Carbonate	2	Cobaltous chloride	.10
Ferrous sulfate	1	Zinc carbonate	.04

[5] 150 mg. thiamine, 150 mg. riboflavin, 500 mg. niacin, 600 mg. calcium pantothenate, 150 mg. pyridoxine and .1lb. B_{12} concentrate (12.5 mg. vitamin B_{12} activity per pound) was added per 100 pounds of feed.

in lots 4 and 5 were similar to that fed in lots 1 and 2 except that solvent soybean meal rather than the blended expeller soybean meal was used. A "special" solvent soybean meal (heated for 30 minutes during which time a final temperature of 230° F. was reached, the 30 minute timing beginning when 212° F. was reached) was used as the protein supplement for pigs in lot 6.

Statistical analysis of the average daily gains using Student's Method with argument "t" as described by Snedecor (1940) was made.

Results and Discussion

The addition of 25 gm. choline chloride per 100 pounds of feed to the basal ration containing either expeller or solvent soybean meal did not improve the rate of gain when a vitamin B_{12} concentrate was included in the ration. Rather, a slight but statistically insignificant decrease in the rate of gain occurred in both instances (lots 2 and 5) when choline was added (table 2). In a previous test, the addition of choline to a similar ration without a source of vitamin B_{12} significantly improved the average daily gains. Since in this test the rate of gain was not improved by the addition of choline, it is suggested that vitamin B_{12} has a sparing effect on the choline requirement of the pig. Similar findings with the rat and chick have been reported by Schaefer *et al.* (1949).

The addition of 21 gm. betaine per 100 pounds of feed did not increase the rate of gain over that produced by the basal ration.

Neither choline nor betaine reduced the amount of feed required to produce 100 pounds gain.

TABLE 2. CHOLINE-BETAINE SUPPLEMENTATION OF A 20% CRUDE PROTEIN CORN-EXPELLER AND SOLVENT SOYBEAN MEAL RATION FOR WEANLING PIGS IN DRYLOT (SPRING 1949)

Items compared	Lot numbers and treatments					
	1	2	3	4	5	6
	Basal (expeller) (inc. 6 B-vit.)[1]	1+250 mg. choline/lb. diet	1+210 mg. betaine per lb. diet	Basal (solvent) (inc. 6 B-vit.)	4+250 mg. choline per lb. diet	Basal special solvent (inc. 6 B-vit.)
No. pigs on test	9	9	9	9	9	9
Av. initial wt., lb.	37.1	37.3	37.6	37.2	37.3	36.8
Av. final wt., lb.	90.0	91.4	86.2	89.5	86.6	94.0
No. da. to reach final wt., days	44.9	50.8	42.8	39.7	38.6	47.2
Av. da. gain, lb. and standard error	1.17± .05	1.07± .03	1.12± .05	1.29± .06	1.26± .06	1.21± .03
Av. daily feed per pig, lb.	3.59	3.67	3.60	4.51	4.09	3.68
Feed eaten per 100 lb. gain, lb.	305	344	317	343	320	304

[1] See [1] Table 1.

The performance of pigs receiving the expeller soybean meal basal ration (lot 1) was slightly inferior to that of pigs fed the solvent soybean meal rations. The rates of gain of each lot of pigs receiving the solvent soybean meal (lots 4, 5 and 6) were very significantly superior to that of pigs in lot 2 ($P \leqq .01$). Since there were no significant differences between the lots receiving the expeller soybean meal and the same was true between lots receiving the solvent soybean meal, the data on average daily gains were combined for pigs receiving each type of soybean meal. The analysis of variance showed that the average daily gains were very significantly more rapid ($P \leqq 0.001$) in the lots fed solvent soybean meal. The average amount of feed required per 100 pounds of gain in each group composed of 3 lots was 322 pounds.

Summary

A 20% crude protein corn-soybean meal ration fortified with minerals, vitamins A and D and 6 vitamins of the B complex (including a B_{12} concentrate) was not improved by the addition of either choline or betaine. Since, in a previous test, choline significantly improved a similar ration without the vitamin B_{12} concentrate it is suggested that B_{12} has a sparing effect on the choline requirement of the pig.

The two solvent soybean meals used were superior to expeller soybean meal, using rate of gain as the criterion, when fed in rations equated to contain 20% crude protein.

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THE BLOOD PROTEINS OF PREGNANT EWES AND HOW THEY ARE AFFECTED BY PROTEIN IN THE RATION[1]

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VERY little information is available on the blood proteins of sheep and especially how they may be influenced by the ration. Woolsey (1913) reported that the blood serum of four normal sheep contained 6.59 grams of total protein per 100 cc. which consisted of 1.15 grams of globulins and 5.44 grams of albumins. Howe (1925) stated that in sheep the albumins predominate over the globulins and that feed has little effect upon them.

The data presented in this paper were obtained in conjunction with a study of the value of different levels and sources of protein in rations for pregnant ewes. The effect of these factors upon lamb production has not been found conclusive, and further studies are in progress. A large number of blood samples have been analyzed for oxyhemoglobin, total serum proteins, albumins and globulins. These data show significant differences between treatments.

Methods and Data

1947–48 Experiment

Forty grade Hampshire ewes were divided into four lots and started on their experimental rations on November 24, 1947. All lots were fed grass hay, yellow corn, bonemeal and a trace mineral salt which contained manganese, iodine, cobalt, iron and copper. The kinds and average amounts of supplement fed per ewe daily for the entire period (November 24, 1947–May 24, 1948) were as follows: Lot I, dried skimmilk, .75 lb.; Lot II, dried skimmilk, .21 lb.; Lot III, dried skimmilk, .05 lb.; and Lot IV, linseed meal, .79 lb. The amount of corn fed was adjusted so that all lots received an equal amount of total digestible nutrients or 2.5 lbs. per ewe daily for the entire period. The amounts of digestible protein fed per ewe per day were: Lot I, .33 lb.; Lot II, .21 lb.; Lot III, .18 lb.; and Lot IV, .33 lb. The ewes were fed the above feeds from three weeks prior to breeding until lambing time with an increase in concentrates made during the last six weeks of pregnancy.

[1] Progress report on Purnell Project No. 137 "A Study of the Effect of Kind and Amount of Protein in the Ration on the Pregnant Ewe and Her Lamb." North Dakota Agricultural Experiment Station. Published with the permission of the Director of the Station.

Blood samples were taken from all ewes at the start of the experiment and every two weeks thereafter. Fourteen blood samples were taken per ewe with a total of 560 samples collected. All samples were analyzed for oxyhemoglobin, total serum proteins, albumins and globulins. Oxyhemoglobin was determined as described by Evelyn (1936). Two ml. of whole blood were diluted to 100 ml. with distilled water. A one ml. aliquot of this solution was diluted to 10 ml. with dilute ammonia water and read in the colorimeter. The method used for the determination of the serum proteins was that of Book and Bolin (1948) which is essentially a modification of the Kingsley method (1939).

The values given (table 1) are averages of 140 samples with their standard errors. The data were treated statistically by analysis of variance as described by Snedecor (1946). Only the differences significant at the 1% level are reported as statistically significant. The mean squares from these analyses are given in table 3 and the least difference between means required for P = 0.01 in table 1.

TABLE 1. OXYHEMOGLOBIN AND SERUM PROTEINS OF EWES FED VARIOUS KINDS AND AMOUNTS OF PROTEIN. (EXPERIMENT 1, 1947–1948)

Lot No.	Supplement	Dig. prot. lb./ewe/da.	Oxyhemoglobin g./100 ml.	Serum total proteins g./100 ml.	Serum albumins g./100 ml.	Serum globulins g./100 ml.
I	dried skimmilk	0.33	10.88±.08	6.95±.04	3.16±.03	3.79±.05
II	dried skimmilk	0.21	11.04±.09	6.92±.05	3.11±.03	3.80±.05
III	dried skimmilk	0.18	11.24±.08	6.75±.04	2.98±.03	3.76±.04
IV	linseed meal	0.33	10.76±.07	6.92±.04	3.20±.03	3.73±.04
Least difference between means required for p=0.01			0.125	0.060	0.043	Not significant

The data in table 1 show the differences in oxyhemoglobin between lots to be significant. These differences were found later, however, to be due to individual ewe variation rather than the ration fed. No previous blood history was used in the allotment of the ewes in this study and a bias between lots was present. The ewes fed the low level of protein had significantly lower total serum proteins than those in the other three lots. This difference was due to a reduction of serum albumins as there were no significant differences in globulins. There was a significant decrease in albumins with each decrease in amount of protein fed. There were no differences between ewes fed dried skimmilk or those fed linseed meal as the protein supplement.

When the ewes in all lots were considered, a significant positive correla-

tion was found between oxyhemoglobin level and serum albumins. This correlation was significant at the 1% point even though the ewes fed the low protein ration had higher than average hemoglobin levels and the albumins were reduced by the ration fed. A significant negative correlation ($r = -0.42$) was found between serum albumins and globulins, that is, ewes with high albumins tended to have lower serum globulins.

1948–49 Experiment

Thirty-six of the ewes that had been used in the 1947–48 study were divided into three lots. An average of the 14 oxyhemoglobin analyses made on each ewe in the previous experiment was used in the allotment of the

TABLE 2. OXYHEMOGLOBIN AND SERUM PROTEINS OF EWES FED VARIOUS KINDS AND AMOUNTS OF PROTEIN. (EXPERIMENT II, 1948–49)

Lot No.	Supplement	Dig. prot. lb./ewe/da.	Oxyhemoglobin g./100 ml.	Serum total proteins g./100 ml.	Serum albumins g./100 ml.	Serum globulins g./100 ml.
I	dried skimmilk	0.33	10.32±.13	7.20±.05	3.19±.04	3.99±.06
II	no supplement	0.19	9.88±.11	7.22±.06	3.03±.05	4.20±.06
III	linseed meal	0.34	10.45±.11	7.40±.06	3.22±.04	4.18±.07
Least difference between means required for P=0.01			0.218	Not significant	.076	Not significant

ewes so that at the start of the study the three lots were uniform as regards to oxyhemoglobin level. All lots were fed grass hay, yellow corn, bonemeal and a trace mineral salt. In addition, Lot I received .64 lb. dried skimmilk per ewe daily and Lot III .68 lb. linseed meal per ewe daily. No protein supplement was fed to the ewes in Lot II. The amounts of digestible protein supplied to each ewe daily were: Lot I, .33 lb.; Lot II, .19 lb.; and Lot III, .34 lb. The amount of corn fed was adjusted so that the ewes in all the lots received 2.6 pounds of total digestible nutrients daily. They were fed these rations from three weeks prior to breeding until lambing time.

All ewes were again bled at two week intervals with a total of twelve samples taken per ewe. The analyses made were the same as those in the 1947–48 experiment. The data obtained in this study are presented in table 2 and the mean squares from the analyses of variance in table 3.

The results of this experiment show a reduction in oxyhemoglobin in ewes fed a ration low in protein. These results are considered to be significant since all lots were uniform in oxyhemoglobin level at the start of the study. No differences in serum total proteins were noted in this year's

investigation, but as in the previous year the serum albumins decreased significantly with reduced amounts of protein fed. Serum globulins were not affected by the ration fed. No differences were found in the blood proteins of ewes fed dried skimmilk as compared to those fed linseed meal as the protein supplement.

Oxyhemoglobin and serum albumins of all ewes in this study were significantly correlated ($r = 0.72$). There was a negative correlation ($r = -0.37$) between serum albumins and globulins.

TABLE 3. TABLE OF MEAN SQUARES

	Degrees of freedom	Oxyhemoglobin	Total proteins	Albumins	Globulins
1947–48 Experiment					
Totals	559				
Between lots	3	6.060*	1.210*	1.460*	.133
Between dates	13	3.707*	1.743*	1.232*	1.260*
Error	543	.921	.209	.105	.274
1948–49 Experiment					
Totals	431				
Between lots	2	13.005*	1.726	1.564*	1.885
Between dates	11	15.657*	2.996*	2.686*	3.362*
Error	418	1.529	.378	.184	.413

* Significant at 1% level.

Discussion

The results of these studies show the importance of obtaining information on the oxyhemoglobin level of the individual sheep prior to the start of an experiment when this factor is used in evaluating the results. The ewes in the 1948–49 experiment were allotted at random as regards to the ration they had received the previous year. Nevertheless, the averages of fourteen oxyhemoglobin analyses made on each ewe in 1947–48 and twelve obtained in 1948–49 were significantly correlated ($r = 0.70$). Thus the oxyhemoglobin level of individual ewes tended to remain constant regardless of ration fed.

Similar analyses were made on the total serum proteins, globulins and albumins. The averages for these constituents for 1947–48 and 1948–49 for each ewe were correlated as follows: total serum proteins, $r = 0.68$; serum globulins, $r = 0.67$; and serum albumins, $r = 0.43$. These correlation coefficients show that ewes with high total serum proteins or serum globulins during one year will tend to have similar levels the following year. Serum albumins are more likely to be affected by years or ration fed.

A study was made of the percent of total variation in oxyhemoglobin

due to differences between ewes within lots in the 1948–49 experiment. The ewes within each of the three lots were placed into groups based on their oxyhemoglobin level as determined in the previous study. This analysis showed that 32 percent of the total variance was due to differences between ewes within lots, 18.9 percent was due to differences between dates when samples were taken and 4.1 percent of the total variance was due to differences between rations fed. Even though the difference between rations proved to be significant this analysis shows the much greater variation that can be expected between individual ewes and between single oxyhemoglobin observations on each ewe.

It will be noted from a study of table 3 that there is consisderable variation in blood proteins between dates when the ewes were bled. During both years and for all blood constituents studied there were significant differences between dates. Thus it would be difficult to measure small differences or changes in the blood proteins of ewes with a limited number of samples from each ewe.

During both years significant positive correlations were found between oxyhemoglobin and serum albumin levels and negative correlations between albumins and globulins. Ewes that had high oxyhemoglobin levels tended to have high serum albumins and low serum globulins.

The results obtained from the two experiments show that the serum albumins of pregnant ewes are affected by the amount of protein fed. Ewes fed rations low in protein tended to have lower serum albumins. The amount of protein in the ration had no apparent effect upon serum globulins. These observations are in agreement with blood protein studies with dogs reported by Zeldis and co-workers (1945), Chow *et al.* (1945), Allison and co-workers (1946) and in other species as stated by Berg (1948). Whether or not the level of protein fed had any influence upon total serum protein is not clear. The reduction of serum albumins in the 1947–48 experiment was sufficient to cause a significant decrease in total proteins but there were no significant differences in the 1948–49 experiment.

The values obtained for serum albumins and globulins are not in close agreement with the limited amount of data on the blood proteins of sheep reported by Woolsey (1913) and Howe (1925). This discrepancy is probably due to a difference in methods in that the 42.5 percent methanol method for the separation of the albumins and globulins was used rather than the sodium sulfate method. Pillemer and Hutchinson (1945) compared these two methods for the separation of albumins and globulins with the electrophoretic method. They found the methanol method was in good agreement with the electrophoretic but the results obtained by the sodium sulfate method were much too high for albumins. In this laboratory the methanol method has been found to give more consistent results than sodium sulfate. This

difference in method used would account for the poor agreement between the values given by Howe and Woolsey and those presented in this paper.

Summary and Conclusions

A total of 992 blood samples of pregnant ewes fed various kinds and amounts of protein were analyzed for oxyhemoglobin, total serum proteins, serum albumins and serum globulins. Considerable variation was found in these constituents between individual sheep and between bi-weekly samples from the same ewe.

Ewes fed rations low in protein had lower serum albumins than those fed a liberal or adequate amount of protein. Low protein rations also caused a reduction in oxyhemoglobin. Serum globulins were not affected by the rations fed.

When fed at an equal level of protein there were no differences in blood proteins between ewes fed linseed meal and those fed dried skimmilk as the protein supplement.

Ewes with high oxyhemoglobin levels tended to have high serum albumins and low serum globulins. In these experiments serum globulins predominated over serum albumins. The oxyhemoglobin, total serum protein and serum globulin levels of individual ewes were highly correlated between years.

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SOME EFFECTS OF TESTOSTERONE PROPIONATE AND THYROPROTEIN IN RAMS[1]

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McKENZIE and Berliner (1937) have shown that under Missouri conditions rams decline in semen production during summer months, or when exposed to high environmental temperatures during other seasons. The deleterious effects of the summer season upon semen quality and production of rams has been found by Green (1940), Phillips *et al.* (1943) and upon that of dairy bulls by Erb, Andrews, and Hilton (1942). Similar results have been obtained through scrotal insulation of rams by Phillips and McKenzie (1934).

Berliner and Warbritton (1937) suggested that a hypothyroid condition may account for impaired semen quality during the summer months. Bogart and Mayer (1946) at Missouri obtained beneficial results by feeding thyroprotein to rams which were showing this so-called "summer sterility." However, Warwick *et al.* (1948) at Washington obtained both deleterious and beneficial results while Eaton *et al.* (1948) at Beltsville found no improvement at all. In view of the conflicting results obtained by these workers, the present study was undertaken to determine the action of thyroprotein upon sexual behavior and semen production under Wisconsin conditions.

Testosterone propionate was also included in this study since the action of exogenous androgen in farm animals has received only slight attention, even though its effect in laboratory animals is well known. In rabbits Cheng and Casida (1949) found increased libido and semen volume, decreased sperm concentration, and no change in total sperm production with testosterone propionate treatment. However, preliminary trials at this Station have failed to show pronounced changes in semen production and libido of rams given testosterone. The possible existence of an interaction between testosterone and thyroprotein in alleviating the symptoms of "summer sterility" was also considered.

Materials and Methods

The group of animals available for this experiment was quite diverse as to age, breed, and condition. Because of this heterogeneity, the animals were

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assigned to treatment groups completely at random. With only a few animals available for each treatment, large random between-group differences were obtained. The change in response from a pretreatment to a treatment period above and beyond the corresponding change obtained in a control group is then inferred to be the consequence of treatment.

An attempt was made to train 24 rams to mount a ewe (held in a breeding crate) and to serve an artificial vagina. Sixteen of the best-trained animals (10 Shropshires, 3 Hampshires, and 3 Oxfords) were finally selected and assigned at random to four treatment groups of four rams each: A. Control (1 Shropshire, 2 Hampshires, 1 Oxford), B. Ten milligrams of testosterone propionate (TP) in oil injected subcutaneously daily (3 Shropshires, 1 Oxford), C. One gram of thyroprotein[2] per hundred pounds of body weight given orally in capsules daily (3 Shropshires, 1 Oxford), D. Ten milligrams TP plus one gram thyroprotein per hundred pounds body weight daily (3 Shropshires, 1 Hampshire). Because of the shortage of space, it was necessary to confine all four animals of each group together rather than in individual pens. The rams were kept in dry lot and fed a ration of alfalfa hay and grain throughout the study.

The experimental interval extended from July 28 to September 25, 1948, and was divided into three periods (Pretreatment, Transitional, and Test) each of twenty days' duration. There were five collection days at four-day intervals in each period. No treatment was given during the first period in order to characterize the level of libido and sperm production of the individual rams. The treatments listed above were applied in the second and third periods. No data were used from the second period in the analysis of results in order to avoid incompletely expressed or transitional effects of treatment.

The rams on semen collection days were led in random turn into the collecting room and allowed a maximum of three minutes to mount the ewe and serve the artificial vagina. Each ram was permitted six trials at approximately thirty-minute intervals. The following observations were recorded for each ram: reaction time (0–180 seconds) per trial, volume of ejaculate per trial, estimated percentage of sperm showing forward motility in each ejaculate, and concentration of sperm in the pooled ejaculates of six trials (haemocytometer count). In addition weekly weights of the animals were taken. Because of the labor involved, it was impossible to test all the rams on the same day. Therefore, one half of the rams from each group were tested on the same day and the remaining animals tested two days later.

[2] The authors wish to express their appreciation to A. G. Engstrom of the Glidden Co. and W. R. Graham, Jr. of the Cerophyl Corp. for supplying the crystalline testosterone propionate and thyroprotein, respectively.

TABLE 1. AVERAGE EFFECTS OF TESTOSTERONE PROPIONATE AND THYROPROTEIN IN RAMS

	A control		B testosterone propionate		C thyroprotein		D TP plus thyroprotein	
	Pretreatment period	Test period minus pretreatment period	Pretreatment period	Test period minus pretreatment period	Pretreatment period	Test period minus pretreatment period	Pretreatment period	Test period minus pretreatment period
Body Weight (pounds)	167	−2	150	−2	152	−11 **A	142	−8 **A,B,C
Reaction Time (seconds)	87	−21	53	−6	23	41 **A	66	−21 **C
Ejaculates (number)	4.27	.80	5.07	.20	4.93	.40	3.67	1.40 *B
Total Sperm (100 millions)	99	46	90	61	95	47	46	78
Volume (mls.)	.46	.17	.52	.26	.43	.23	.20	.35 **A
Concentration (10,000/mm.³)	307	62	373	−97 **A	384	−19	346	9
Sperm motility (% showing forward motion)	44	−17	35	−14	65	−24	37	−10

* −P<.05
** −P<.01
A indicates difference when compared with group A.
B indicates difference when compared with group B.
C indicates difference when compared with group C.

Results and Discussion

While sixteen rams were carried on the experiment, four (one Shropshire from each group) have been excluded from all analyses except that of weight because of missing data on semen production. One ram had an attack of bloat and for a time exhibited abnormal responses. A second ram failed to serve the vagina early in the experiment and was replaced by an alternate too late for complete results from the latter individual. A third produced semen devoid of sperm, apparently due to a bilateral obstruction in the ducti deferentia near the ampullae since sperm were demonstrated in the testes and lower ducti deferentia at autopsy. The fourth ram lost considerable weight, showed marked symptoms of thyrotoxicosis, and eventually ceased to serve the vagina.

TABLE 2. ANALYSIS OF VARIANCE OF REACTION TIME—CONTROL VS. THYROPROTEIN—3 RAMS PER GROUP

Source of variation	D/F	Mean square
Period	1	380
Day/Period	8	186
Group	1	6552
Ram/Group	4	4066
Period×Group	1	5246**
Ram/Group×Period	4	868
Day/Period×Group	8	223
Day/Period×Ram/Group	32	140
Total	59	672

** Highly significant at the 1% level.

Weight

Considerable variation appeared in the weekly weights of the animals but all groups showed average losses in weight during the course of the experiment. Significant differences were not obtained between groups A (Control) and B (TP), table 1. (Use was made of the analysis of variance technique illustrated in table 2; the mean square for the interaction Group × Period was tested against the error mean square, Ram within Group × Day within Period, table 2.) However, both groups receiving thyroprotein (C and D) showed highly significant weight losses when compared with the controls (table 1). Also a small but highly significant difference was obtained between groups C and D. It would appear that TP partially counteracted the loss in weight produced by thyroprotein, although the mechanism of such an action is at present unknown. Kochakian and Murlin (1935, 1936) and Kochakian (1937) have shown that testosterone increases nitrogen retention in castrated dogs, whereas Andrews, Beeson, and Harper (1949) have demonstrated that testosterone implants caused increased weight gains in wether lambs, apparently due to increased fat deposition.

Reaction Time

Reaction time can be considered a measure of libido; the greater the time, the less the libido or sex drive. The control group showed a diminishing reaction time throughout the experiment (table 1). Group B showed less of a decrease although there was no significant difference between these groups. Thyroprotein alone produced a very pronounced increase in reaction time. As in body weight, TP appeared to counteract this deleterious effect of thyroprotein when administered together in group D. This point is further brought out in the highly significant difference between groups C and D.

However, it must be borne in mind that the data for this analysis are based on only three rams, the one being excluded in group D that failed to serve following the onset of thyrotoxicosis. If TP does have some counteraction to thyroprotein, it was not apparent in this one ram.

An incidental observation in the course of the experiment showed that the reaction time within any one collection day increased in all rams at an almost uniform rate from the first trial (mean—30 seconds) to the sixth trial (mean—90 seconds).

Number of Ejaculates

One aspect of the expression of libido is the number of completed ejaculates on a given collection day. From a possible six were subtracted the number of refusals (those trials in which the ram had not ejaculated within the prescribed 180 second interval) and the number of "blank" ejaculates (those completed attempts in which no measurable amount of semen was recovered).

All groups showed an increase in the number of ejaculates during the course of the experiment. No significant differences between groups appeared except in the comparison of B and D, the latter group showing a much greater increase in this characteristic (table 1).

Total Sperm

The number of sperm ejaculated by an individual is determined by two variables, the volume of semen and the concentration of sperm cells in the semen. Moreover, volume is in part controlled by the libido of the individual insofar as the number of ejaculates is concerned.

All groups showed considerable increases in total sperm production in the progress of the experiment. However, no significant differences were obtained between these groups (table 1). It may be concluded, then, that none of the treatments had an appreciable effect on the rate of sperm production. But by breaking total sperm into its two direct components, some treatment effects can be observed.

A. Volume—All groups again showed increases, but only group D showed a significantly greater increase than that of the controls (table 1).

In analyzing these results on a trial rather than a day basis, it has been confirmed (McKenzie and Berliner, 1937) that the second ejaculate is greater in volume than the first, the volume of subsequent ejaculates declining in a rather constant manner.

B. Concentration—The control group increased moderately in concentration of sperm cells during the experiment. Groups C and D underwent

very little change with treatment. On the other hand, group B demonstrated a pronounced decline in concentration (table 1).

Sperm Motility

A drop of each ejaculate was diluted with modified Kreb's solution (Lardy and Phillips, 1942) on a microscopic slide and examined at 450× under a cover slip to estimate the percent of sperm showing forward motility. Only the first measurable ejaculate produced by each ram on each collection day was used in the analysis. All groups showed a decline in sperm motility as the experiment progressed, but no significant differences could be found between groups (table 1).

General Discussion

The control rams in the course of the experiment showed a slight loss in weight, decreases in reaction time and sperm motility, and increases in number of ejaculates, total sperm, semen volume, and sperm concentration. These changes may be a result of continued training or of seasonal variation or both. The experimental period extended from the low point of the year as regards semen quality (McKenzie and Berliner, 1937) to the onset of the breeding season when one would expect to find rams approaching their maximum capacities. The improvement seen in the control group may therefore be in part a reflection of the season. An attempt was made to correlate the various characters under test with several expressions of the weather, particularly humidity and temperature, but no significant associations could be found. Mean daily temperatures in Madison for the months of July, August, and September were 73.8, 72.4, and 65.6° F. respectively.

Since it was necessary to confine all the rams of each group together rather than individually, some precision in the measurement of both libido and semen characters may have been lost because of ranting behavior. Moreover, any pen differences in environment, if they did exist, are included in the group differences and cannot be removed from the treatment effects.

Results of this study are not in line with the work of Bogart and Mayer (1946) inasmuch as no beneficial results were obtained through the use of thyroprotein, while actually deleterious effects were demonstrated on libido and body weight. However, Wisconsin summer temperature is possibly lower than that found in Missouri. TP, likewise, appeared not to improve sperm production or sperm motility; sperm concentration was reduced but volume increased sufficiently that there was a consequent insignificant change in total sperm when compared with the controls. By combining TP with thyroprotein, however, the effects of the latter were partially avoided in loss of body weight. TP also may have partially prevented the loss in

libido caused by thyroprotein, and the combined treatment resulted in increased volume. TP, however, failed to protect one ram from losing all libido when it developed thyrotoxicosis.

Summary

Four groups of three rams each were studied on the following treatments during the interval from July to September, 1948: A. Control, B. Ten milligrams of testosterone propionate injected subcutaneously daily, C. One gram of thyroprotein per hundred pounds body weight daily, D. Ten milligrams of testosterone propionate plus one gram of thyroprotein per hundred pounds body weight daily. The control group demonstrated a slight loss in body weight and some decrease in sperm motility during the course of the experiment. There were also increases in sexual libido, total sperm, semen volume, and sperm concentration as the study progressed. The rams treated with testosterone, when compared with the controls, showed a significantly greater decrease in sperm concentration but changes in the other characters did not differ significantly. The group receiving thyroprotein showed a significantly greater loss in body weight and increase in reaction time than the controls. Semen volume, sperm concentration and motility, and total sperm were not significantly affected. Treatment with a combination of testosterone and thyroprotein resulted in a significantly greater weight loss than in the controls, but less than in the thyroprotein group. There was also a greater increase in semen volume, but changes in sperm concentration, motility, and total sperm did not differ significantly from the controls. There was some indication that testosterone may prevent the lowering of libido caused by thyroprotein.

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THE COMPARATIVE VALUE OF HYDRAULIC, EXPELLER AND SOLVENT PROCESSED OIL MEALS FOR RUMINANTS[1]

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THE similarity in nutritive value of hydraulic processed cottonseed, peanut and soybean oil meals was demonstrated in two earlier studies (Briggs *et al.*, 1946 a, 1947). The meals were used singly and in combination to supplement the protein of prairie hay in rations for steers and lambs. The digestibility of nutrients in the rations and the amounts of nitrogen retained by the animals while on these rations served as a basis for comparison. The present experiments, also carried out with steers and lambs, were undertaken to determine whether or not different methods of commercial processing of oil meals affected their nutritive value for ruminants.

In nitrogen balance trials with lambs Miller and Morrison (1944) found that the protein furnished by two solvent-process soybean oil meals which had received different heat treatments had a higher digestibility than that furnished by the raw beans. This difference in digestibility of protein largely accounted for a greater retention of nitrogen by lambs when fed the solvent- process oil means than when fed the raw-soybean ration. Biological values for the protein of these different soybean products were found to be about the same. In experiments with dairy heifers, Gallup and Kuhlman (1931) found that heating hydraulic cottonseed meal in an autoclave for 30 minutes under 25 pounds of steam pressure decreased the apparent digestibility of the protein. Other investigations relating to the present research were discussed in previous papers (Briggs *et al.*, 1946a, 1947).

Materials and Methods

The oil meals used for comparison were cottonseed meal produced by hydraulic and solvent processes, and soybean oil meal produced by expeller and solvent processes. The four meals were commercial products obtained at the same time in amounts sufficient for all experiments.[4] Complete details of the method of preparation were not available. According to the manufacturer different batches of meal may be given slightly different heat treatment in processing but as a rule temperatures of 275° F. are attained for a short time in preparation of expeller soybean oil meal whereas a temperature

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of about 235° F. is maintained for about an hour in processing hydraulic cottonseed meal. In the preparation of solvent soybean oil meal the soybeans are extracted with very little heat but the extracted flakes are then moistened and toasted at about 233° F. for 30 to 60 minutes. Solvent cottonseed meal receives the least heat treatment, there being a short period of time in flash drying when the temperature may reach 225° F.

The average composition of the four oil meals used in the present study is shown in table 1.

TABLE 1. AVERAGE COMPOSITION OF OIL MEAL SUPPLEMENTS AND OTHER FEEDS USED IN MAINTENANCE- AND FATTENING-TYPE RATIONS FOR STEERS AND FOR LAMBS

Animals fed	Feed description	Dry matter	Composition of dry matter				
			Ash	Crude protein	Ether extract	Crude fiber	N-free extract
		percent	percent	percent	percent	percent	precent
Steers	C. S. Meal, hydraulic	92.51	6.78	46.48	5.68	8.52	32.54
Steers	C. S. Meal, solvent	91.35	6.70	44.48	2.58	11.60	34.64
Steers	S. B. O. Meal, expeller	90.81	6.77	51.76	5.02	5.98	30.47
Steers	S. B. O. Meal, solvent	90.92	6.95	55.85	0.61	5.37	31.72
Steers	Ground yellow corn	89.94	1.62	10.65	3.75	1.96	82.02
Steers	Prairie hay	92.13	7.22	4.43	2.46	33.48	52.41
Lambs	C. S. Meal, hydraulic	92.18	6.65	45.06	6.16	8.42	33.71
Lambs	C. S. Meal, solvent	91.60	6.80	43.04	2.94	11.82	35.40
Lambs	S. B. O. Meal, expeller	91.36	6.72	50.67	5.11	5.59	31.91
Lambs	S. B. O. Meal, solvent	91.18	6.89	54.11	0.63	5.03	33.34
Lambs	Ground yellow corn	87.08	1.55	10.23	3.54	1.88	82.80
Lambs	Prairie hay	92.40	7.12	4.67	2.74	33.79	51.68

Both of the soybean meals were higher in protein and lower in crude fiber than either of the cottonseed meals. This difference in protein, however, is not necessarily characteristic of the two products. The solvent soybean oil meal contained less than 1 percent of oil whereas the solvent cottonseed meal contained over 2 percent of ether-soluble material. The larger amount of ether-soluble material in solvent cottonseed meal is attributed to difficulties encountered in the commercial extraction of cotton seeds.

The comparative nutritive value of the four meals was determined in digestion and nitrogen balance trials with 4 steers and 8 wether lambs. The meals were incorporated in rations as supplements to a constant amount of prairie hay in maintenance-type rations and as supplements to a constant amount of ground corn and prairie hay in fattening-type rations. The protein content of all rations was equalized at about 10 percent by varying the amount of each oil meal supplement. No allowance was made for differences among similar rations with respect to carbohydrate and fat content since

TABLE 2. AVERAGE DAILY AMOUNT AND COMPOSITION OF DRY MATTER IN MAINTENANCE- AND FATTENING-TYPE RATIONS PREPARED WITH DIFFERENT OIL-MEAL SUPPLEMENTS FOR STEERS AND FOR LAMBS

Ration type and oil meal supplement	Daily dry-matter intake		Composition of total dry matter intake				
	Total	Oil meal supplement	Organic matter	Crude protein	Ether extract	Crude fiber	N-free extract
RATIONS FOR STEERS							
	grams	grams	percent	percent	percent	percent	percent
Maintenance							
C. S. Meal, hydraulic	4307	562	92.86	10.10	2.85	30.94	48.97
C. S. Meal, solvent	4318	573	92.89	9.89	2.45	31.29	49.26
S. B. O. Meal, expeller	4227	482	92.89	10.00	2.73	31.03	49.13
S. B. O. Meal, solvent	4186	441	92.86	9.86	2.24	31.20	49.56
Fattening							
C. S. Meal, hydraulic	6205	438	96.48	11.40	3.53	10.74	70.81
C. S. Meal, solvent	6243	476	96.50	11.48	3.32	10.96	70.74
S. B. O. Meal, hydraulic	6146	379	96.51	11.40	3.47	10.64	71.00
S. B. O. Meal, solvent	6123	356	96.50	11.51	3.22	10.62	71.15
RATIONS FOR LAMBS							
Maintenance							
C. S. Meal, hydraulic	609	78	92.74	9.20	3.09	30.39	50.06
C. S. Meal, solvent	611	80	92.73	9.02	2.66	30.70	50.35
S. B. O. Meal, expeller	600	68	92.72	9.25	2.91	30.40	50.16
S. B. O. Meal, solvent	593	62	92.70	9.13	2.42	30.60	50.55
Fattening							
C. S. Meal, hydraulic	835	62	96.65	11.45	3.65	10.90	70.65
C. S. Meal, solvent	848	67	96.70	11.47	3.08	11.46	70.69
S. B. O. Meal, expeller	843	54	96.65	11.47	3.39	11.08	70.71
S. B. O. Meal, solvent	840	51	96.66	11.55	3.11	11.09	70.91

these differences were small and comparable to those that would be produced in practical feeding. Miller and Morrison (1944) found that the replacement of the oil removed in the preparation of solvent soybean oil meal had no effect on nitrogen retention.

The daily fattening rations for steers contained 1820 grams of hay and 4540 grams of corn in addition to the oil meal supplements. Salt was fed *ad lib.* and 15 grams of Na_2HPO_4 were added to the soybean meal maintenance-type rations. The lambs were fed amounts of hay and concentrates which constituted about 14 percent of the corresponding rations fed the steers. In addition, the lambs received daily 14 grams of NaCl and 0.75 gram of fortified codliver oil. Mixtures of NaH_2PO_4 and $CaCO_3$ were added to the salt in amounts necessary to meet the requirements of lambs (Pearson *et al.*, 1945). The composition of the maintenance and fattening rations exclusive

of the mineral additions is shown for the steers and for the lambs in table 2.

The steers were yearling Herefords weighing about 480 pounds at the beginning of the trials. The wether lambs were Texas feeder lambs which weighed about 57 pounds. The animals were kept in individual metabolism stalls with provisions made for the separation and quantitative collection of feces and urine as described elsewhere (Briggs and Gallup, 1949). They were given a constant amount of their respective rations twice daily during 20-day periods, feces and urine being collected during the last 10 days of each period. Aliquots of the daily collections of feces and urine were stored in tightly closed glass jars in a refrigerator at approximately 0° C. The urine was acidified with hydrochloric acid and a small amount of thymol was added to the feces samples to aid in preservation. Nitrogen was determined on the feces previous to drying for proximate analysis (Gallup and Hobbs, 1944).

The schedule of feeding the maintenance-type rations in the trials with steers was so arranged that during any one trial each of the four steers received a different ration until all steers had received the four rations. The same schedule was then followed in feeding the fattening rations. There were no feed refusals.

A similar schedule was followed in feeding the maintenance-type rations to lambs until all eight lambs had received the four rations. In the fattening trials following the maintenance trials with lambs this order of feeding was not always maintained since there were occasional feed refusals which made it necessary to repeat some trials. Between trials and during preliminary feeding periods the lambs were kept in large false-bottom metabolism cages.

Results and Discussion

During the trials when the maintenance-type rations were fed the steers made an average gain of 0.9 pound per day and the lambs maintained slightly more than their original weight. When changed to the fattening rations the steers and lambs made average daily gains of 2.3 pounds and 0.30 pound, respectively.

The results of the digestion and nitrogen balance trials with both steers and lambs are presented in table 3.

There were no marked differences in nutrient digestibility between similar rations made up with the two differently processed cottonseed meals or between similar rations made up with the two differently processed soybean oil meals. The general trend of digestibility values with the possible exception of that for ether extract favored the soybean oil meal concentrates in both maintenance and fattening rations for both steers and lambs.

It is of significance perhaps, that for both steers and lambs the digestibility of all nutrients except crude fiber was greater in the fattening rations

TABLE 3. DIGESTIBILITY OF NUTRIENTS IN MAINTENANCE- AND FATTENING-TYPE RATIONS CONTAINING DIFFERENT OIL MEAL SUPPLEMENTS AND THE PERCENTAGE OF NITROGEN RETAINED BY STEERS AND BY LAMBS FED THESE RATIONS

Ration type and oil meal supplement	Apparent digestibility of—						Total nitrogen intake (daily)	Nitrogen in feces (daily)	Nitrogen in urine (daily)	Nitrogen retained (daily)
	Dry matter	Organic matter	Crude protein	Ether extract	Crude fiber	N-free extract				
RESULTS WITH STEERS										
	percent	percent	percent	percent	percent	percent	grams	grams	grams	percent
Maintenance										
C. S. Meal, hydraulic	62.3	65.1	56.7	58.8	71.2	63.3	69.8	30.1	26.4	18.2
C. S. Meal, solvent	60.8	63.4	56.6	52.2	68.7	62.1	68.5	29.6	27.3	16.9
S. B. O. Meal, expeller	63.6	66.2	63.6	56.9	72.6	63.3	67.8	24.6	32.0	16.5
S. B. O. Meal, solvent	63.2	65.9	61.4	46.5	73.0	63.3	66.2	25.5	29.5	16.9
Fattening										
C. S. Meal, hydraulic	73.8	75.1	63.4	76.3	56.6	79.7	112.8	41.4	36.5	30.9
C. S. Meal, solvent	72.1	73.5	60.3	72.1	57.9	77.7	114.3	45.5	32.5	31.7
S. B. O. Meal, expeller	75.5	76.8	64.3	71.6	62.6	81.3	111.7	40.0	36.6	31.4
S. B. O. Meal, solvent	75.6	77.2	65.5	71.0	65.3	81.2	112.4	39.7	37.1	31.7
RESULTS WITH LAMBS										
Maintenance										
C. S. Meal, hydraulic	57.3	60.1	54.7	58.0	64.7	58.5	8.97	4.07	3.65	13.9
C. S. Meal, solvent	57.4	60.2	54.3	49.9	64.2	59.5	8.82	4.03	3.62	13.3
S. B. O. Meal, expeller	58.3	61.4	60.8	51.5	65.5	59.4	8.87	3.44	4.29	13.0
S. B. O. Meal, solvent	58.8	61.9	61.1	41.5	66.8	59.9	8.67	3.35	3.84	17.1
Fattening										
C. S. Meal, hydraulic	77.3	79.5	66.1	78.6	54.6	85.5	15.30	5.18	7.07	20.0
S. C. Meal, solvent	76.1	78.3	63.3	75.7	55.0	84.4	15.61	5.70	6.95	18.7
S. B. O. Meal, expeller	78.6	80.9	69.2	75.0	60.3	86.1	15.46	4.77	7.86	18.3
S. B. O. Meal, solvent	79.2	81.3	69.6	74.5	60.8	86.7	15.54	4.73	7.73	19.8

than in the maintenance rations. The digestibility of crude fiber in the maintenance ration was about 71 percent by steers and about 65 percent by lambs, and in the fattening rations about 61 percent by steers and 58 percent by lambs. Although these two types of rations differed in their amount and composition of total dry matter, by far the greater part of the fiber in both types was supplied by the prairie hay. The decreased digestion of this fiber, when the fattening rations were fed, is believed to be related to the activity and type of organisms predominating in the rumen in the presence of large amounts of readily fermentable carbohydrates. Corn was the source of this carbohydrate in the fattening ration. The effect of pure carbohydrate on the digestibility of dry matter and other nutrients in roughage and mixed rations has been observed in recent work at Pennsylvania (Swift *et al*, 1947) and Ohio (Burroughs *et al.*, 1949). The value of the protein supplements in increasing the digestibility of fiber in prairie hay has been pointed out in previous publications (Briggs *et al.*, 1947) (Gallup and Briggs, 1948).

The oil meal supplements supplied approximately 60 percent of the total nitrogen in the maintenance rations and about 28 percent of that in the fattening rations. Consequently, relative differences between the oil meals in quality of proteins, as measured by nitrogen retention values, might logically be expected to be the more pronounced in the results obtained with the maintenance-type rations. The nitrogen retention values shown in table 3, however, failed to reveal any significant difference between the oil meals when used in either type of ration.

It is noteworthy in this connection that in the trials with steers the fattening rations which supplied daily about 1.6 times as much nitrogen as the maintenance rations permitted nitrogen retention (grams, daily) over three times that obtained with the maintenance rations. Although differences in composition of the two types of rations prevent complete interpretation of these results, increased feed intake and the nitrogen-sparing action of carbohydrates may account for the more efficient utilization of the nitrogen in the fattening rations. Protein nitrogen is wasted when carbohydrates are lacking in a ration. It is an interesting possibility that this wastage occurs in feeding for maintenance under practical conditions and that under such conditions protein can be replaced in part by carbohydrates without effecting an unfavorable change in the nitrogen balance of the animal. If true, such a replacement would effect considerable saving of protein in feeding supplements to steers under range conditions.

In the trials with lambs, the fattening rations supplied about 1.7 times as much nitrogen as the maintenance rations. Nitrogen retention on the fattening rations (grams, daily) was over twice that on the maintenance rations.

Summary

The comparative value of cottonseed meal prepared by hydraulic and solvent processes and soybean oil meal prepared by expeller and solvent processes was determined in digestion and nitrogen balance trials with steers and lambs. The meals were fed on an equivalent protein basis in amounts which furnished about 60 percent of the total nitrogen in maintenance-type rations of prairie hay and oil meal and about 28 percent of the total nitrogen in fattening rations of corn, prairie hay and oil meal. All rations contained about 10 percent of protein.

Differences among the meals attributed to differences in processing were not evident in the results.

The digestibility of nutrients was higher in the soybean oil meal rations than in the cottonseed meal rations of similar type for both steers and lambs. The cottonseed meal rations and the corresponding soybean oil meal rations were of equal value, however, in promoting nitrogen retention. The protein of the low carbohydrate maintenance rations was poorly utilized. The average percentages of total nitrogen intake retained by steers on the maintenance-and fattening-type rations were 17.4 and 31.5, respectively, and by lambs on these rations 14.4 and 19.2 respectively.

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RAT FEEDING TESTS WITH MILK OBTAINED FROM COWS FED TOXIC COTTONSEED MEAL

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PROPERLY processed cottonseed oil meal has been accepted as a suitable protein supplement for livestock feeding since the beginning of the present century. It was soon recognized however that raw cottonseed meal could not be fed to certain animals such as the pig, chicken, rat, mouse, guinea pig and rabbit, but required special processing to relieve it of toxic constituents. This toxicity was generally attributed to the presence of gossypol and its related derivatives which occur in concentrations approximating 1% of the cottonseed kernel. More recently however, several investigators have indicated a lack of correlation between the toxicity of the raw or improperly processed meal and its content of gossypol pigments. Boatner *et al.* (1948) demonstrated that all of the physiologically deleterious components of cottonseed are segregated in the pigment glands which constitute approximately 2–4% of the cottonseed kernel. It was found that feeding pigment glands to chicks at a level of 0.65% of a soybean diet resulted in depressed growth, reduced food efficiency and a lowered percent of livability. Even though feeding pure gossypol did result in significantly inferior growth, the deleterious affect produced by either the raw meal or pigment glands was much greater than that of gossypol alone. Eagle (1948) repeated these observations with rats, mice, guinea pigs and rabbits.

The ruminating animal appears to be able to tolerate the toxic principles in raw cottonseed meal as evidenced by numerous feeding tests (Kuhlman *et al.*, 1936, Hoffman *et al.*, 1929, Cunningham *et al.*, 1934 and Reed *et al.*, 1928). To our knowledge, however, no one has determined whether or not the milk obtained from cows receiving "toxic "cottonseed meal contains the toxic factors. Numerous examples can be cited for the transmission of toxic materials into milk, one of the most recent being DDT (Telford *et al.*, 1945). Since significant quantities of improperly processed or "toxic" cottonseed meal are still being fed to dairly cattle, it is pertinent to know whether these toxic factors can be transmitted into the milk thereby presenting a potential threat to the well being of the person or animal consuming the milk.

[1] The authors are grateful to Mr. C. G. Meeks for his technical assistance and to Dr. F. A. Norris of Swift & Company for the procurement and preparation of the hydraulic and hexane extracted cottonseed meal samples used in these tests.

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Experimental

Cow Feeding Test

Two cows (one Guernsey and one predominantly Brown Swiss) were obtained a few days after calving for use in this experiment. Three test diets were prepared, each diet being fed to both cows for a period of one week. During the control period or first week, a soybean meal concentrate was fed. This regimen had the following percentage composition: soybean oil meal 45, wheat bran 20, cane molasses 15, alfalfa meal 10, pulverized oats 5 and mineral supplement 5. Since the level of protein in the concentrate was abnormally high, timothy hay was fed *ad libitum*. The amount of concentrate fed was based upon the amount of milk produced, an attempt being made to feed 1 kilogram of concentrate for each two kilograms of milk produced.

TABLE 1. SUMMARY OF THE AMOUNTS OF CONCENTRATE AND HAY CONSUMED AND THE AMOUNT OF MILK PRODUCED BY EACH COW DURING EACH FEEDING PERIOD

Cow	Total concentrate consumed	Total hay consumed	Total milk produced[1]	Kg. milk per kg. concentrate	Kg. milk per kg. meal[2]
Period 1—Feeding soybean meal concentrate					
Guernsey	37.6	35.4	93.3	2.48	5.51
Brown Swiss	40.3	43.2	111.1	2.75	6.10
Period 2—Feeding hydraulic cottonseed meal concentrate					
Guernsey	58.1	47.3	101.5	1.75	3.90
Brown Swiss	40.3	50.4	107.9	2.68	5.95
Period 3—Feeding hexane extracted cottonseed meal concentrate					
Guernsey	52.2	48.2	95.4	1.83	4.07
Brown Swiss	36.3	53.6	106.2	2.92	6.50

[1] In the case of the Guernsey cow, a mechanical injury to the right front teat made it impractical to collect the milk from this quarter. Therefore, the figures reported for the amount of milk produced by the Guernsey cow are only an approximation obtained by measuring the amount of milk collected from the other three quarters and adding 1/6 of this amount to make the total.

[2] Soybean or cottonseed meal.

During the second week of the test, the two cows were given a ration identical to that used during the first week except for the substitution of hydraulic cottonseed meal for the soybean meal. This sample of meal was free from toxicity as evidenced by rat feeding tests.

An identical procedure was followed in the third week except that both cows received a hexane extracted cottonseed meal[3] in place of the soybean

[3] A pilot plant product expecially prepared by continuous extraction with commercial hexane in metal equipment. No attempt was made to detoxify the meal by cooking or chemical treatment.

oil meal. This meal was shown to be very toxic when fed to weanling rats.

After each change in diet, the first two days' milkings were discarded, and the milk collected during the remaining five days was heated to 110° F. homogenized, placed in conventional paraffined milk cartons and frozen and stored at −10° F. for use in the rat feeding test. An accurate record was kept of the amount of concentrate and hay consumed and the amount of milk produced by each cow for each test period. (table 1).

Rat Feeding Test

To determine whether the milk obtained from cows fed hexane (toxic) cottonseed meal was toxic when compared to the milk obtained during the periods in which the soybean and hydraulic cottonseed meals were fed, the milk samples were fed to an animal known to be sensitive to cottonseed toxicity; namely, the male weanling rat. To make the test extremely critical, the rats were fed a milk diet supplemented only with vitamins and minerals. Since Withers *et al.* (1913, 1917) have shown that certain iron salts will destroy the toxic principles in cottonseed meal, adding iron to milk might

TABLE 2. GROWTH, FOOD CONSUMPTION AND FOOD EFFICIENCY DATA OBTAINED WITH RATS RECEIVING THE MILK SAMPLES

Group No.	Regimen	Avg. wt. gain per rat per 6 wks.—gm.	Avg. cc. of milk used per rat per 6 weeks	Cc. milk used to produce 1 gm. wt. gain
1.	Milk from the Brown Swiss cow receiving soybean meal	186±25	2540 cc.	13.6 cc.
2.	Milk from the Guernsey cow receiving soybean meal	187±20	2250	12.0
3.	Milk from the Brown Swiss cow receiving hydraulic cottonseed meal	170±15	2545	14.9
4.	Milk from the Guernsey cow receiving hydraulic cotton seed meal	177±19	2565	14.4
5.	Milk from the Brown Swiss cow receiving toxic hexane extracted cottonseed meal	178±12	2546	14.3
6.	Milk from the Guernsey cow receiving toxic hexane extracted cottonseed meal	178±16	2534	14.2
7.	Composite milk from both cows receiving cottonseed meal, with 1 mg. % of pigment glands added to the milk	All dead by the second week		

destroy any toxicity present. Therefore, 0.5 mg. iron as iron chloride, 0.05 mg. copper as copper sulfate and 0.05 mg. manganese as manganese sulfate per day were administered to all rats bi-weekly by dropper. In a similar manner, two drops of of A and D oil (3000 I.U. of vitamin A and 400 I. U. of vitamin D per gram) were administered to each rat twice a week.

Six groups of male weanling rats (eight rats per group) were housed in individual cages in a room in which the temperature and humidity were carefully controlled. These animals received the six milk diets described in table 2. Since all of the toxicity associated with cottonseed meal is concentrated in the pigment glands, a seventh group of animals was included in the test to demonstrate this toxicity in the presence of milk. The diet received by group 7 therefore consisted of milk supplemented with 1 mg. % of pigment glands.

The milk samples were thawed and fed fresh daily, all unconsumed food being measured and discarded on the following day. In the case of diet 7, the pigment glands were homogenized into the milk by means of a Waring Blendor. The animals were weighed at weekly intervals and an accurate record of the amount of milk consumed was maintained for the eight week experimental period. Due to the fact that the animals grew at a slower rate during the last two weeks of the test, the data collected during the first six weeks were used for calculating the food efficiency values (table 2). The average weights of the animals at 8 weeks were: group 1, 277 gm., group 2, 260 gm.; group 3, 249 gm.; group 4, 245 gm.; group 5, 256 gm., and group 6, 260 gm. All groups started at the same average initial weight (40 gm.).

Discussion

It should be pointed out that this test was rigidly designed in order that the results obtained would be conclusive. Accordingly, a high protein ration was fed in order that large amounts of the toxic substances would be consumed by the cows. To further accentuate the test, an attempt was made to maintain a ratio of 1 kilogram of concentrate fed for every 2-3 kilograms of milk produced.

Both cows appeared to remain in good health when fed the toxic cottonseed meal. Nor could any off-flavor be detected in the milk obtained during the week in which the hexane extracted cottonseed meal was fed

Fat and protein analyses were obtained for the milk samples, and were found to average 4.08 % fat and 3.32% protein for the Brown Swiss and 5.08% fat and 3.56% protein for the Guernsey cow. The slightly higher fat content of the Guernesy milk may have accounted for the slightly better growth observed in the case of rats receiving this milk (table 2).

The rat feeding tests were rigidly carried out in that the rats were given

nothing but the milk diets supplemented with vitamins and minerals. Furthermore, the milk obtained during the last day of each test period was fed first since it was thought that the level of toxic constituents should be at a maximum in this milk.

From the data presented in table 2, it may be concluded that the toxic substances which the cows consumed in the form of hexane extracted cottonseed meal, were not transmitted into the milk in sufficient quantity at least to affect the growth rates or the food efficiencies of the male weanling rats receiving the milk. Assuming the level of pigment glands to be at least 2% of the hexane cottonseed meal, and employing the data presented in table 1, 1, 4.92 and 3.08 gm. of pigment glands were consumed by the Guernsey and Brown Swiss cows respectively for each kilogram of milk produced. If the toxic principles in the pigment glands had been transmitted completely into the milk, the milk would contain at most the equivalent of 492 and 308 mg. % pigment glands. In table 2 (group 7), it can be seen that 1 mg. % of pigment glands was sufficient to produce toxicity and resulting death in male weanling rats. Therefore it would appear that the toxicity was not transmitted into the milk since not even a fraction (1/492 or 1/308) of the activity of the pigment glands consumed by the cows could be detected in the milk by feeding it to rats. It would also appear that no appreciable amounts of gossypol were transmitted into the milk since none of the appetite depressing effects associated with gossypol (Zucker *et al.*, 1947 and Eagle 1949) were seen in the rats. Since approximately 50% of the pigment gland is gossypol, relatively large amounts of gossypol were consumed by the cows. Even though the milk appeared to be free of any toxicity as tested with the rat, this does not preclude the possibility that still other factors may exist in the milk which might provoke an allergic reaction particularly in the human consuming the milk.

Summary

A sample of hexane extracted cottonseed meal which was known to be toxic to male weanling rats, was fed to two cows for a period of one week. The milk collected from these cows was fed to weanling male rats to determine whether significant amounts of the toxic substances occurring in the cottonseed meal had been transmitted into the milk. Feeding the milk as an exclusive diet to weanling rats for a period of eight weeks failed to reveal any indication of toxicity. Milk containing a very low level of the toxic constituents on the other hand was found to be toxic to rats of the same age. Therefore it can be concluded that the toxicity associated with improperly processed cottonseed meal cannot be transmitted into the milk of the dairy cow receiving the toxic meal. This does not preclude the possiblity that still

other factors may be transmitted into the milk which might provoke an allergic reaction particularly in the human consuming the milk.

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THE INFLUENCE OF ALFALFA HAY AND FRACTIONS OF ALFALFA HAY UPON THE DIGESTION OF GROUND CORNCOBS[1,2]

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IN a previous report (Burroughs *et al.* 1949b) corn starch was shown to exert little or no influence upon the dry matter digestion of good quality alfalfa hay. Corn starch, however, when added to a poor quality roughage (corncobs) resulted in a marked decrease in roughage dry matter digestion. Since approximately the same levels of protein were fed in each roughage series, the two different results could best be explained on the basis that alfalfa hay contained superior non-protein nutrients essential for the growth of rumen micro-organisms involved in roughage digestion. It was postulated that rumen micro-organisms fermenting starch required certain non-protein nutrients aside from energy for their growth and development. Also, rumen micro-organisms involved in roughage digestion required the same or similar unknown nutrients. Since starch is largely devoid of nutrients other than energy; the roughage must therefore supply these unknown nutrients for both the starch and the roughage. Starch fermentation proceeds faster than roughage digestion. Consequently, if a roughage is fed with starch its digestibility will not be lowered if it has ample quantities of these unknown nutrients to supply the requirements of micro-organisms subsisting on both the starch and the roughage. Conversely, if insufficient quantities of these unknown nutrients are present in a roughage, then starch will depress roughage digestion since the faster acting starch attacking micro-organisms will rob the roughage of nutrients needed for the slower acting micro-organisms concerned in roughage digestion.

The purpose of this paper is to present data indicating the presence of nutrients in alfalfa hay which exert a favorable influence upon the digestion of a poor quality roughage such as corncobs when fed to cattle.

Experimental Procedure and Data

Three series of digestion trials were carried out using grade Hereford steers, 2 to 3 years of age. In the first series, corncob digestion was determined in rations containing variable amounts of alfalfa hay. The second series included comparisons of corncob digestion in rations with and without addi-

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tions of a water extract of alfalfa hay. During the third series the influence of alfalfa ash upon upon corncob digestion was studied.

The methods and analysis used in each digestion experiment were essentially the same as those employed earlier (Burroughs *et al.*, 1949a and Burroughs *et al.*, 1949b). Ten-day collection periods on a given ration were used with 4 steers preceded by an adjustment period of 10-days or longer on the same ration. A slight variation in this procedure was used in the water-extract-of-alfalfa series. In that series digestion measurements were made throughout both the adjustment and regular collection periods.

Digestible organic matter as well as digestible dry matter was determined, but since the results of these two determinations were essentially the same, only the dry matter values are presented. Sufficient quantities of the various feeds were secured at the start of an experiment to last throughout a given series.

The corncobs were obtained from a local elevator, cleaned of all adhering grain and husks, and ground in a hammer-type mill. The alfalfa hay in the first series of trials was good second cutting, having a protein content of 14.5 percent. It was chopped to approximately 2-inch lengths to facilitate weighing and handling. The corn starch was a finely divided power manufactured primarily for laundry use. Crude casein was used to regulate the protein content of certain rations as will be noted later.

Roughage digestion with variable ratios of alfalfa hay and corncobs

The rations containing different ratios of alfalfa hay and corncobs are presented in table 1. A total of 5 pounds of roughage per steer per day was fed in each ration. The first ration contained the minimal amount of alfalfa hay (.5 pound) which would result in the ready consumption of a large amount of corncobs (4.5 pounds). The second ration was made up of 1 pound of alfalfa and 4 pounds of corncobs. The third ration contained equal amounts of the two roughages and the fourth ration contained 4 pounds of alfalfa hay and 1 pound of corncobs. Five pounds of alfalfa and no corncobs were fed in the fifth ration.

Each of the first five rations contained 4.0 pounds of corn starch per steer daily, 0.2 pound bone meal, 0.1 pound salt, 10 ml. of high potency vitamin A and D feeding oil, and sufficient crude casein to maintain an approximately constant protein level in all rations. The sixth ration in this series was similar to the all-alfalfa hay ration (No. 5) except for the omission of corn starch. This ration was included to determine whether or not starch inhibited the digestibility of the particular alfalfa hay being used. A seventh ration consisted of 4 pounds of corncobs and 1 pound of alfalfa hay without starch. It was included for purposes of measuring the maximum digestion of corncobs under more-or-less ideal feeding conditions.

The apparent coefficients of digestion of the roughage dry matter as pre-

sented in table 1 were corrected on the basis that 8 percent of the starch dry matter was undigestible (Burroughs *et al.*, 1949a).

The apparent coefficient of digestion of the total roughage in ration 1 containing the least amount of alfalfa hay was the poorest value of all of the roughage combinations fed, averaging 41.8 percent. Increasing the alfalfa hay to 1 pound per steer daily, (ration 2 table 1) resulted in a marked increase in total roughage digestion averaging 49. 4 percent. A further increase in the alfalfa content in the roughage mixture to 2.5 pounds increased the average roughage digestion to 56.8 percent. This was followed by a fur-

TABLE 1. CORNCOB DIGESTION WITH DIFFERENT AMOUNTS OF ALFALFA HAY

Description, feeds and animals	Trial No.						
	1	2	3	4	5	6	7
Amounts (lbs.) fed daily per steer							
Alfalfa hay (chopped)	0.5	1.0	2.5	4.0	5.0	5.0	1.0
Corncobs (ground)	4.5	4.0	2.5	1.0	—	—	4.0
Starch	4.0	4.0	4.0	4.0	4.0	—	—
Casein	0.6	0.5	0.3	0.1	—	—	—
Bone meal	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1
A and D feeding oil (ml.)*	10	10	10	10	10	10	10
Apparent Coefficient of digestion of roughage dry matter							
Steer 28	52.8	55.9	62.7	62.6	63.0	59.8	61.9
Steer 33	42.1	53.3	57.6	58.0	64.0	60.2	58.6
Steer 34	46.4	50.8	60.0	60.0	58.7	61.6	58.5
Steer 35	26.0	37.5	57.0	58.1	62.5	61.9	56.6
Average	41.8	49.4	56.8	59.7	62.0	60.9	58.9
Computed apparent coefficient digestion of corncobs assuming Coefficient of digestion of alfalfa hay constant at 60.9							
Average	39.7	46.5	52.7	54.9	—	—	58.4

* 800 international units of vitamin D and 3000 I.U. vitamin A per ml.

ther increase in digestion to (59.7 percent in ration 4 (table 1) in which 4 pounds of alfalfa hay were fed in the roughage mixture.

The increased total roughage digestion with increased amounts of alfalfa hay might be explained on the basis that alfalfa hay has a digestibility markedly superior to that of corncobs even when each roughage is fed under conditions promoting their maximum respective digestibility. No such large difference actually existed. Feeding alfalfa hay or corncobs along or in combinations in the absence of corn starch has previously been shown to produce

maximum digestibility in either a good or poor quality roughage (Burroughs *et al.*, 1949a). The alfalfa hay in this series when fed as the sole roughage either with or without starch (rations 5 and 6) was digested on the average between 61 and 62 percent. Corncobs when fed without starch and with a minimum of alfalfa hay was digested only slightly less (58.4%—ration 7 table 1) than the alfalfa hay.

Since the two roughages under rather ideal conditions of digestion, (absence of starch), were digested almost equally well, the observed differences in the digestion of the roughage combinations when fed along with corn starch must be accounted for in some other way than a difference in their potential maximum digestion. If one assumes that the alfalfa hay under all conditions was being digested to a constant maximum amount then it is possible to compute the digestion of corncobs when fed with starch and the variable amounts of alfalfa hay.

These computed values for corncobs are presented as averages in table 1. With the least amount of alfalfa hay, corncobs were digested 39.7 percent. Each increment of alfalfa hay in the respective rations resulted in increased digestion of corncobs. The largest alfalfa addition resulted in a calculated digestion coefficient of 54.9 percent for the corncobs. This value approaches without equalling the maximum observed digestion of corncobs (58.4 percent) in ration 7 (table 1) when no starch was included in the ration.

Influence of water extract of alfalfa upon corncob digestion

The water extract of alfalfa was prepared from dehydrated alfalfa meal containing 17 percent protein. Sufficient tap water was added to the meal to make a thin gruel. This was allowed to stand 15 minutes and the liquid pressed out using a large lard press. The process was repeated three times. The liquids obtained were combined and concentrated by evaporation in large shallow pans before a fan in a room heated to 90° F. The concentrated liquid appeared as a heavy dark syrup containing only enough moisture to allow its ready mixture with the other ration ingredients.

The basal ration (table 2) contained 4 pounds of corncobs, 2.88 pounds of corn starch, 1.6 pounds of dried skimmilk, .32 pound bone meal, .1 pound salt and 10 ml. of a high potency vitamin A and D feeding oil per steer per day. Four grade Hereford steers about two years of age received this ration approximately one month prior to the time they were placed in digestion stalls. Digestion of corncob dry matter with this basal ration was determined initially in a 10-day collection period. The digestibility averaged 34.4 percent (table 2).

The steers were then given the alfalfa extract added to the basal ration. Each steer received daily the extract from 4 pounds of alfalfa meal. Digestion measurements on this ration were begun immediately following the feeding of the basal ration without removing the steers from the digestion stalls

TABLE 2. INFLUENCE OF WATER EXTRACT OF ALFALFA UPON CORNCOB DIGESTION

Description, feeds and animals	Trial No.						
	1	2	3	4	5	6	7
	Amounts (lbs.) fed daily per steer						
Corncobs (ground)	4.00	4.00			4.00		
Starch	2.88	2.88			2.88		
Dried skimmilk	1.60	1.60			1.60		
Bone meal	.32	.32			.32		
Salt	.10	.10			.10		
A and D feeding oil (ml.)*	10	10	Repetition of period 2	Repetition of period 2	10	Repetition of period 5	Repetition of period 5
Extract of alfalfa	—	+			—		
	Apparent coefficient digestion corncob dry matter						
Steer 26	34.4	39.0	42.7	46.5	55.8	26.3	38.6
Steer 27	37.0	41.0	49.2	55.6	54.1	37.4	44.0
Steer 28	36.4	29.3	47.1	45.5	44.2	34.1	20.7
Steer 29	29.8	32.1	45.4	47.9	39.4	39.8	43.0
Average	34.4	35.4	46.1	48.9	48.4	34.4	36.6

* 800 International units of vitamin D and 3,000 I.U. vitamin A per ml.

for a customary 10-day adjustment period. Three successive 10-day digestion trials were made while feeding the alfalfa extract.

During the first 10-day period which customarily would represent the adjustment period, corncob digestion (average (35.4 percent) was increased little if any by the extract addition. During the second 10-day period, corncob digestion was increased materially averaging 46.1. The digestion of corncobs during the third 10-day period averaged 48.9 percent or very similar to the values obtained in the second 10-day period.

The steers were then returned to the basal ration without the alfalfa extract and 3 successive 10-day digestion measurements were made using no intermediate adjustment period. Corncob digestion remained high (average 48.4 percent) during the first 10 days following the feeding of alfalfa extract and then dropped to 34.4 and 36.6 percent respectively during the two succeeding 10-day periods. These latter values closely approximated the initial digestibility of 34.4 percent observed with corncobs prior to the feeding of alfalfa extract.

Influence of alfalfa ash upon corncob digestion

The influence of alfalfa ash upon corncob digestion was determined by feeding a basal ration containing corncobs and comparing it with a similar ration to which alfalfa ash was added. The ash was prepared by first burning dehydrated alfalfa meal in long smoldering piles and then ashing at dull red

heat in a muffle furnace. The amount of alfalfa ash fed each steer daily was approximately the amount of ash secured from 4 pounds of alfalfa meal which was also the amount of corncobs fed daily to each steer.

The rations and the apparent coefficients of digestion of corncob dry matter are presented in table 3. Trials 1 and 2, without and with alfalfa ash, are comparable in that the corncobs used in these two trials were from a common source. Digestion of corncobs was materially increased in the

TABLE 3. INFLUENCE OF ALFALFA ASH UPON CORNCOB DIGESTION

Description, feeds and animals	Trial No.				
	1	2	3	4	5
Amounts (lbs.) fed daily per steer					
Corncobs (ground)	4.00	4.00	4.00	4.00	4.00
Starch	2.88	2.88	2.88	2.88	2.88
Dried skimmilk	1.60	1.60	1.60	1.60	1.60
Bone meal	.32	—	.32	—	.32
Salt	.10	.10	.10	.10	.10
A and D feeding oil (ml.)*	10	10	10	10	10
Alfalfa ash	—	.32	—	.32	—
Apparent coefficient digestion corncob dry matter					
Steer 26	33.7	46.3	11.2	41.9	20.6
Steer 27	46.8	54.8	39.1	43.2	55.3
Steer 28	33.6	52.7	35.6	49.5	35.5
Steer 29	39.8	54.1	40.4	39.3	27.5
Average	38.5	52.0	31.6	43.5	34.7

* 800 international units of vitamin D and 3,000 I.U. vitamin A per ml.

ration containing alfalfa ash (average 52.0 percent) as compared with the basal ration which average 38.5 percent.

The corncobs used in trials 3, 4, and 5, (table 3), were from a second source and represent a repetition of the results obtained in trials 1 and 2. Corncob dry matter digestion in the initial basal ration (period 3 table 3) average 31.6 percent. The feeding of alfalfa ash increased corncob digestion to 43.5 percent. Upon returning the steers to the basal ration, corncob digestion dropped to 34.7 percent. Each digestion period in this series of trials was preceded by a 10-day or longer adjustment period.

Discussion

The results obtained in this study further substantiates the results obtained earlier (Burroughs *et al.*, 1949b) that alfalfa hay contains a nutrient(s)

in addition to protein which in some way protects it from being digested to a lesser extent when fed in the presence of starch. This nutrient(s) appears to be contained in sufficient amounts in alfalfa so that when a poor quality roughage is fed such as corncobs in conjunction with alfalfa, the poor quality roughage is also benefited.

The nature of this unknown nutrient(s) would appear to be partially or totally soluble in water and in part or totally associated with the inorganic constituents contained in alfalfa hay. The evidence thus far presented can best be explained on the basis that the unknown material(s) under consideration in alfalfa hay is an essential nutrient(s) for rumen micro-organisms involved in roughage digestion. This view is further strengthened by *in vitro* roughage digestion studies (unpublished data) with rumen micro-organisms. The addition of alfalfa ash and certain complex mineral mixtures definitely increases the ability of rumen micro-organisms to digest cellulose as well as roughage dry matter under controlled laboratory conditions.

The importance of these findings to livestock production in addition to their academic interest would seem to lie in the possibility that poor quality roughages may need special mineral supplementation. Also the probability exists that quality in roughages as it applies to livestock feeding may be dependent to a large extent upon the mineral make-up of the roughage in supplying nutrients for rumen microorganisms involved directly in roughage digestion or utilization.

Summary

Three series of digestion trials with 4 grade Hereford steers 2 to 3 years of age were used in measuring the influence of alfalfa hay and fractions of alfalfa hay upon corncob digestion. Corncob digestion was improved progressively with four respective additions of alfalfa hay. A water extract of dehydrated alfalfa meal or the ash of the alfalfa meal fed at the rate equivalent to 4 pounds meal daily per steer improved corncob digestion materially.

The results are interpreted to indicate the presence of inorganic nutrients in good quality alfalfa hay or meal which are essential to rumen micro-organisms involved directly in roughage digestion. These findings were discussed with respect to their importance in roughage utilization and livestock production.

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THE INFLUENCE OF CASEIN UPON ROUGHAGE DIGESTION IN CATTLE WITH RUMEN BACTERIOLOGICAL STUDIES[1,2]

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CERTAIN high-protein feeds have been shown in this Laboratory to exert favorable influences upon the digestion of poor quality roughages fed to cattle. Soybean oil meal (Burroughs *et al.*, 1949a) increased the dry matter digestibility of both timothy hay and corncobs when fed in a fattening-type steer ration. Dried skimmilk (Burroughs *et al.*, 1949b) increased the digestibility of certain roughages when corn starch made up a part of the ration. These favorable influences of protein-rich feeds may have resulted from their supplying either or both protein and non-protein nutrients essential to rumen micro-organisms concerned in roughage digestion. Gallup and Briggs (1948) increased the dry matter digestion of prairie hay with cottonseed meal. Watson *et al.* (1947) on the contrary failed to note an increase in digestibility of a mixed hay with protein supplementation. The purpose of this study was to determine the influence of protein *per se* upon roughage digestion and to observe the effects of different feeding regimes on rumen micro-organisms.

Experimental Procedure

The choice of a basal ration low in protein and yet palatable enough to be readily consumed by cattle offered somewhat of a problem. Corncobs represents a roughage with a low protein content (less than 2 percent). Corn starch was a suitable carbonaceous feed with a low amount of protein (less than 2 per cent). It was included in the basal ration since starch had been shown (Burroughs *et al.*, 1949c) to increase the need for dried skimmilk and presumably protein in maintaining efficient roughage digestion.

Earlier attempts to get cattle to consume sufficient quantities of combinations of corncobs, corn starch, and casein supplemented with a simple mineral mixture and vitamin A and D feeding oil had failed. This ration became palatable only when dried skimmilk was substituted for the casein (Burroughs *et al.*, 1949b). The use of dried skimmilk in the present study, however, was not entirely satisfactory since it contained other nutrients in addition to protein. Further studies (unpublished data) revealed that the

[1] Published with the approval of the Director of the Ohio Agricultural Experiment Station.
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addition of small quantities of high quality alfalfa hay to a corncob-starch-casein ration made it sufficiently palatable for digestion measurements.

The basal ration, (ration 2 table 1) contained 4 pounds of corncobs, 1 pound of chopped alfalfa hay, 4 pounds of mineralized corn starch, .1 pound salt, and 10 ml. of vitamin A and D feeding oil per steer per day. The palatability of this ration although satisfactory was extremely low in that it required a second 10-day collection period before complete feed consumption was attained and digestion measurements could be made at one time with all four experimental animals. This ration contained 3.4 percent protein (Nx6.25).

Preceding the feeding of the basal ration, a similar ration was fed which differed only with respect to the omission of corn starch. It was included for comparative purposes in confirming earlier work (Burroughs *et al.*, 1949b) demonstrating the influence of starch in lowering the digestion of the roughage mixture. Also, direct observations were desired as to the rumen bacteria which such a ration would support in comparison with similar rations containing starch with and without casein additions. The protein content of ration 1 was 4.6 percent (Nx6.25).

Ration 3 contained 11.1 percent protein (Nx6.25) an increase of nearly 8 percent protein over the basal ration (No. 2 table 1) which resulted from the feeding of 1 pound of casein per steer per day. The fourth and final ration contained 2 pounds of casein per steer daily which further raised the total ration protein content to 17.4 percent (Nx6.25). The palatability of all these rations, except No. 2, was good and no difficulty was experienced in getting the cattle to consume their entire feed regularly.

The details of the digestion measurements were essentially the same as those described earlier (Burroughs *et al.*, 1949b). Ten-day collection periods with 4 steers 3 years of age were made, preceded by adjustment periods of 10-days or longer on the same ration. Apparent coefficients of dry matter digestion were determined as a measure of total ration digestibility. Total roughage digestion was computed from total ration digestibility upon the basis that 12.8 percent of the mineralized starch was undigested (Burroughs *et al.*, 1949b).

Rumen samples for bacteriological examinations were obtained with a large bore stomach tube at the end of each digestion period. These examinations included the total number of micro-organisms present in the rumen of each steer on the 4 respective rations. Gram stain observations were also made in studying the morphological types of micro-organisms present. In addition, certain cultural studies were made of the bacteria which served as an indication of their physiological characteristics. The details of the bacteriological technics were similar to those reported earlier (Gall *et al.*, 1949).

Results

Digestion

Roughage dry matter digestion in ration 1 (table 1) containing no starch was good, averaging 60 percent. The addition of corn starch to this ration (ration 2) resulted in a severe lowering in roughage digestion to an average

TABLE 1. THE INFLUENCE OF CASEIN UPON ROUGHAGE DIGESTION

Description, feeds and animals	Ration No. and percent protein in ration			
	1 4.6	2 3.4	3 11.1	4 17.4
Amounts (lbs.) fed daily per steer				
Corncobs (ground)	4.0	4.0	4.0	4.0
Alfalfa hay (chopped)	1.0	1.0	1.0	1.0
Starch (mineralized)[1]	—	4.0	4.0	4.0
Casein (commercial)	—	—	1.0	2.0
Salt	0.1	0.1	0.1	0.1
Vitamin A & D feeding oil (ml.)[3]	10	10	10	10
Apparent coefficient of digestion of roughage dry matter				
Steer 28	59.5	14.9[2]	40.7	57.2
Steer 33	60.9	19.3[2]	46.3	51.5
Steer 34	59.3	10.4	51.2	51.7
Steer 35	60.5	8.0	47.3	53.5
Average	60.0	13.2	46.4	53.5

[1] The mineralized starch contained 90 percent corn starch and 10 percent bone meal.

[2] Satisfactory feed consumption and digestion measurements were made earlier on ration 2 with steers 28 and 33 in which the digestion values obtained were 18.2 and 15.6 respectively.

[3] The vitamin A and D feeding oil contained 800 international units of vitamin D and 3000 international units of vitamin A per ml.

of only 13.2 percent. By adding one pound of casein, which increased the ration protein to 11.1 percent, (ration 3) roughage dry matter digestion increased sharply to an average of 46.4 percent. Increasing the ration protein to 17.4 percent (ration 4) increased roughage digestion somewhat more, averaging 53.5 percent. This latter value approaches without equaling the digestibility obtained in ration 1 in which no starch was fed.

Whether additional amounts of casein would have increased roughage digestion further is not known. Judging from other results, (unpublished data) it seems most logical to assume that the limiting factor(s) in roughage digestion in ration 4 was not protein *per se* but rather factor(s) which could have been supplied by additional amounts of alfalfa hay.

Bacteriological observations

The results of the bacteriological studies are presented in table 2. The total number of rumen bacteria observed in slide counts averaged 49.6 billions per gram of wet solid ingesta when the steers received ration 1. This value is of the magnitude observed previously (Gall *et al.*, 1949) when cattle and sheep received farm-type rations. The addition of starch (ration 2) resulted in a severe drop in rumen bacterial population to an average of 24.8 billions per gram. This decrease in total number of rumen organisms paralleled the decrease noted in roughage digestion with ration 2 (table 1).

Upon adding casein (ration 3) the total number of observed rumen bacteria averaged 47.1 billion per gram. This is a substantial increase over ration 2 containing no casein; and the increased number of rumen bacteria coincides with a substantial increase in digestibility of the roughage (ration 2, table 1). A slightly larger number of rumen bacteria were noted with the largest addition of casein, however, the difference observed between rations 3 and 4 was not statistically significant.

The predominating morphological types of bacteria as seen on Gram stains were markedly different on the various rations. The organisms observed while the animals received ration 1 were very similar to those described previously (Gall *et al.*, 1949) in which cattle received farm-type rations. The rumen bacteria observed when ration 2 was fed had changed sufficiently so that the Gram stains from this period could be sorted out from the Gram stains of other periods without the aid of identifying marks. Some of the types of bacteria noted while the animals received ration 1 disappeared entirely or were present in very reduced numbers. A large rod shaped bacterium not previously seen made its appearance.

Further changes in the bacterial picture shown by Gram stains were observed (ration 3) when the rod seen on slides from animals fed ration 2 was no longer present, and long chains of cocci appeared in large numbers. Slides from steers fed ration 4 were characterized by the disappearance of most of the cocci in chains as seen on ration 3, while cocci in masses took their place. Most of the bacteria seen on slides from animals fed ration 1 had reappeared with the feeding of ration 4.

Differences in both kinds and numbers of cultured bacteria were apparent as the steers were fed different rations. The bacteria cultured were arbitrarily placed in two broad groups designated as fast-growers or slow growers depending upon whether they grew in less than 48 hours or whether they required a longer period for growth. The fast-growing cultures usually produced a marked turbidity and lowered the pH of the culture media, while the slow-growing organisms frequently clumped cotton placed in the media and did not lower the pH.

The pattern of growth obtained from samples of rumen contents from

steers fed ration 1 was similar to that found on low grain winter rations fed to cattle as reported previously (Gall *et al.*, 1949b). About one half of the cultures isolated were slow-growing rods, which clumped the cotton and did not produce much acid, while the other half were fast growing cocci, which lowered the pH markedly and produced turbidity.

TABLE 2. RESULTS OF RUMEN BACTERIAL STUDIES WITH CASEIN ADDITIONS TO A LOW-PROTEIN RATION

Factors studied	Ration number							
	1		2		3		4	
Average bacterial count (Billions/gram)	49.6±17.1		24.8±5.6		47.1±2.0		48.4±9.5	
Average % dry matter of rumen sample	24.6± 2.5		25.0±2.6		26.3±2.3		26.4±0.9	
pH range of rumen samples	7.1– 7.5		7.2–7.8		6.2–6.5		5.8–6.2	
Percent cultures producing gas	53		14		54		53	
Type of cultural growth*	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow
Number of cultures grown	8	7	12	2	15	7	14	5
Number cultures producing a pH of:								
5.5 or below	8	0	12	1	10	1	10	0
5.6 or above	0	6	0	1	5	6	4	5
Number cultures of:								
Cocci	8	0	4	1	11	1	13	0
Rods	1	7	8	1	7	7	8	5
Pleomorphic rods	0	0	5	1	0	1	0	0
Flora clumping cotton	0	7	0	1	0	6	0	4

* Cultures growing within 48 hours were regarded as fast growers whereas those taking a longer time were classified as slow growers.

The cultural results from ration 2 were decidedly different from those of ration 1 although about the same number of cultures grew in both periods. Eighty-three percent of the growth occurred in 48 hours in period 2, (table 2). Only one of these cultures clumped the cotton and it failed to lower the pH of the media. In contrast to the large number of cocci found on ration 1, only 4 cultures of fast growing cocci were isolated from the same steers fed ration 2. Six of the nine remaining cultures were large pleomorphic rods which were probably the organisms seen in large numbers on the Gram stains for this period.

The cultural results from the rumen contents of steers fed ration 3 were different from the results obtained when the same animals were fed ration 1 or 2. A higher total growth was obtained; with the increased growth being mostly due to the larger numbers of slow-growing rods in case of ration 3. More cocci were cultured in ration 3 than in 2, and many mixed cultures

occurred. The pleomorphic rod so often isolated in ration 2 was found only once in ration 3.

The cultural results from the animals fed ration 4 was somewhat different from ration 3, but the changes between these two periods were less marked than between any of the others. There was a slight decrease in growth in this period and the reduction was due to the decrease in the number of the slow-growing organisms isolated. The number of cultures of rods and cocci were about the same as in the preceding period, and many mixed cultures occurred. More large gas-producing cocci occurred in cultures from ration 4 than on any of the other rations, and no pleomorphic rods characteristic of ration 2 were found.

Discussion

The results obtained in this study indicate that protein *per se* exerts a favorable influence upon roughage digestion when roughages are fed to cattle in rations containing starch. This substantiates earlier findings (Burroughs *et al.*, 1949a and 1949b) in which soybean oil meal and dried skimmilk respectively increased roughage digestion.

The need for a minimal amount of protein in cattle rations for maximum roughage digestion seems apparent. No attempt was made in this study to determine this minimal protein level, however, it would appear that this level at times must exceed 11 percent protein which was the amount fed in ration 3. This figure also coincides with an estimated minimal level of protein for efficient roughage utilization as found in other studies (Burroughs *et al.*, 1949b).

The importance of protein *per se* to roughage digestion when a roughage is fed alone or in the absence of a starchy feed seems small indeed. The results obtained in this study with ration 1 were similar to those obtained earlier indicating as little as 4 or 5 percent protein in a roughage is ample for good digestion. Apparently the protein requirement for efficient roughage digestion decreases as starch or starchy grains are reduced in the ration. This should not be inferred to mean that this small amount of protein in a roughage is ample in meeting the body needs of cattle since requirements for roughage digestion and body needs are distinct and not additive even though ration protein jointly serves the two requirements. It would appear most logical to compute protein requirements of cattle upon the basis of the larger single need for this nutrient. That is, if ration protein supplying the body needs of cattle (maintenance, growth, etc.) exceeds the needs of protein for roughage digestion, then the requirement should be based solely upon the minimum protein needed in covering body needs without any consideration as to the amount of ration protein needed for efficient roughage digestion. If the reverse relationship exists in which the protein needs for

roughage digestion exceeds the body protein requirement, then protein requirements of cattle should be determined solely upon the basis of the minimal amount required for maximal roughage digestion.

The bacteriological findings lend support to the theory proposed earlier (Burroughs *et al.*, 1949c) that the method by which protein aids roughage digestion is by furnishing an essential nutrient for rumen bacteria concerned directly in roughage digestion. On this basis the protein requirements for roughage digestion become the protein (nitrogen) requirements for rumen bacterial growth. In the present studies, whenever sufficient protein was fed and roughage digestion was good, satisfactory numbers of rumen bacteria were present and the predominating types differed morphologically and culturally from those found when roughage digestion was poor.

Summary

Various additions of casein to a low-protein cattle ration containing roughage and corn starch resulted in increased dry matter digestibility of the roughage part of the ration.

Rumen bacteriological studies with and without the casein additions revealed differences which correlated closely with the degree of roughage digestion. These studies included counts of the rumen bacterial populations, Gram stain morphology observations, and cultural work pertaining to the physiology of the rumen bacteria present.

The results offer further evidence of the need for a minimum protein requirement for efficient roughage digestion in cattle, especially when starch or starchy grains make up a part of the ration. This minimum protein requirement is interpreted as representing a protein (nitrogen) requirement for the growth of rumen micro-organisms directly involved in roughage digestion.

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COBALT DEFICIENCY IN NEW YORK STATE

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FROM time to time during the past several years, reports have filtered into the College of an unhealthy condition of cattle in the northern counties of New York State which clearly could not be diagnosed as deficiencies of phosphorus, calcium or a lack of total feed. As information concerning cobalt deficiency became more abundant, many points of similarity were recognized between the symptoms of these New York cattle and the described symptoms of cobalt deficiency. Chemical analysis of forage samples from so-called "trouble farms" by one of us (KCB) gave support to the cobalt deficiency hypothesis in that many forage samples ran critically low in this element. Several veterinarians in the northern areas, particularly Dr. Grace of Potsdam, cited remarkable responses in some unhealthy cattle when cobalt salts were administered as a drench.

In 1947 a study of cobalt metabolism in sheep was initiated having as its purpose the biological testing of forage crops from selected areas of the State for cobalt adequacy and furthermore to determine the metabolic role of cobalt in the animal body, particularly the ruminant. The present paper is a report from this study.

No attempt will be made here to review the literature extensively since several excellent reviews of cobalt nutrition are available. Among the more recent are those by Russell (1944) and Maynard and Smith (1947).

Methods and Materials

Lambs were chosen as the test animal in this study since the literature indicates that they are more sensitive to a lack of cobalt than cattle and because of their smaller size, larger numbers can be handled within a given budget. Western feeder lambs purchased from the stockyards were freed of parasites by dipping and drenching and carefully inspected for signs of other disease. They were then placed in pens for individual feeding of a cobalt-low ration.

Two experiments are here reported extending over a period of about 2 years. The general procedure in both of these studies was to feed all lambs within an experiment a low-cobalt ration until the majority gave evidence of cobalt deficiency. At this time the lambs were randomized into treatment groups and the study continued. The first experiment involved 16 lambs which were randomized into two groups—(A) continued on a cobalt-low

ration and (B) the same ration supplemented with orally administered cobalt salts. The second experiment involved 21 lambs randomized into three treatment groups: (1) cobalt-low ration; (2) cobalt supplemented orally and (3) cobalt supplemented by intramuscular injections.

The hay fed in both experiments was secured from New York farms that had past reports of trouble in cattle. The first experiment utilized hay from a farm in St. Lawrence County. It unofficially graded as a No. 3 mixed grass hay and averaged 0.04 ppm of cobalt. In the second experiment hay, unofficial grade No. 3 mixed grasses, was secured from a farm in Madison County. Cobalt analyses made at intervals during the trial ranged from 0.02 to 0.05 ppm. In both experiments the hay was supplemented with locally purchased shelled corn, which ranged in cobalt concentration from 0.01 ppm to 0.03 ppm and milk powder (Borden's Klim) which by analysis contained 0.01 ppm of cobalt or less. Lambs which would take a full ration were fed at the rate of about 1 pound of hay, 1.25 pounds of shelled corn and 0.25 pound of milk powder per day. Half of the ration was fed in the morning and half in the afternoon. As appetites failed due to a deficiency of cobalt, much lower quantities of these feeds were consumed as is indicated later. Plain block salt and tap water were generally available to all lambs.

As appetites failed, more feed was presented to the lambs than they would clean up in 2–3 hours. This was purposely done to be certain that lambs were being fed all that they would take since feed intake is one of the important criteria of cobalt deficiency. Accurate records of the daily feed intake were kept for each lamb. Cobalt supplements were administered in the first experiment as the sulfate salt and in the second experiment as the chloride salt. In all cases, unless otherwise stated, cobalt was administered twice per week at the rate of 1 mg. of cobalt per lamb per day. Cobalt contamination was rigorously guarded against since lambs will respond to very minute amounts of this element.

The feed samples were analyzed for cobalt by the method of Ellis and Thompson (1945). Hemoglobin was determined on jugular blood samples by the oxyhemoglobin method (Sheard and Sanford, 1929). Blood serum calcium was determined by the Clark-Collip method and inorganic phosphorus by the Fiske-Subbarow technic. Blood serum proteins were determined with the gravity-gradient column (Lowry and Hunter, 1945) after the method had been satisfactorily checked with Kjeldahl-determined proteins (Chibnall *et al.*, 1943) on large samples of sheep serum.

All lambs that died or were sacrificed were carefully autopsied[1] and tissues taken for microscopic study. These tissues consisted of the liver, spleen, kidney, pituitary gland, thyroid gland and sections at various levels

[1] Courtesy of Dr. P. Olafson of the New York State Veterinary College.

of the intestinal tract. The tissues were fixed in Susa's fixitive and stained with hematoxylin and eosin. In a few cases some tissues were also stained to demonstrate hemosiderin by the Prussian blue reaction.

Red blood cells were counted in the conventional way using a 1:400 dilution blood pipette. Centrifuge hematocrits were determined using the modified centrifuge tube previously described (Smith 1944).

Results

Experimental

The first gross symptom noted in the lambs as they became cobalt deficient was a waning appetite. This was followed by reduced rates of gain and then

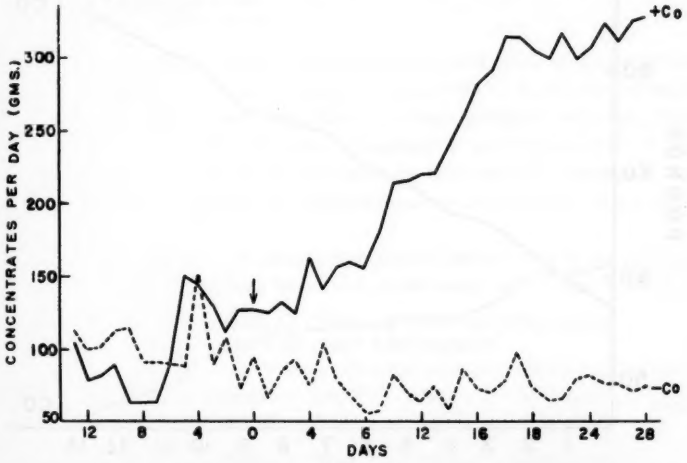

Figure 1. The daily consumption of concentrates of cobalt-deficient and cobalt-fed lambs. The arrow indicates the start of cobalt supplementation.

weight losses. A progressive anemia occurred some time later. These symptoms have been described by others (Russell 1944).

In our studies a cobalt deficiency did not develop until after some months on the low-cobalt ration. In the first experiment cobalt deficiency was definitely recognized at about 5 months and in the second experiment at about 7 months and at these times treatments were begun.

The inappetence in cobalt deficiency and the rapid response of appetite following oral administration of cobalt salts is well illustrated in figure 1. The intake of concentrates (corn plus milk powder) only is shown in this figure. A similar response occurred in the intake of hay but to a lesser degree. It is noted that feed intake showed an increase over basal conditions at about

the sixth day after cobalt feeding. In the second experiment appetite responded definitely at about the eighth day after cobalt feeding. To all outward appearances a failing appetite is a dominant symptom of cobalt deficiency. Unfortunately this symptom is not specific for cobalt inadequacy. All other gross symptoms could well be secondary to the inappetence. There have been some reports of a depraved appetite in cobalt deficiency. This symptom has not been marked in our lambs. In the two experiments, only 2 lambs gave evidence of this by chewing the wool off of other lambs. While there was ample opportunity for the lambs to chew wood from the

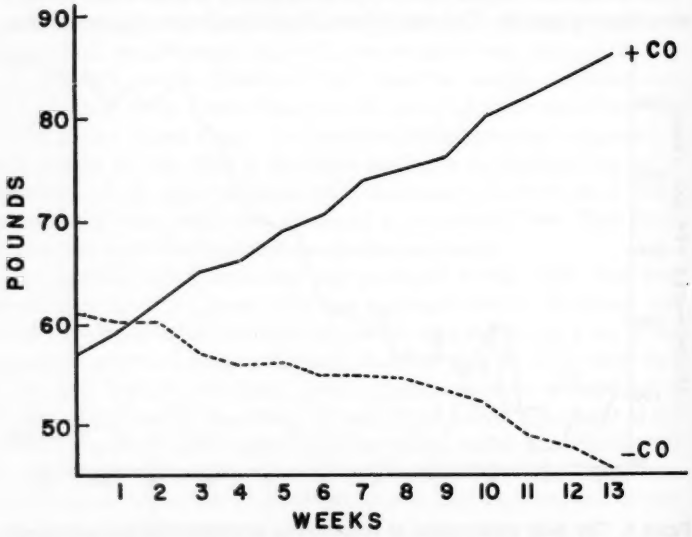

Figure 2. The weekly weight gains of cobalt-deficient and cobalt-fed lambs.

pens or to eat the sawdust bedding, no real evidence of this was observed.

Growth curves of the lambs in the first experiment are presented in figure 2. The progressive decline in body weight of those fed the cobalt-low ration only and the normal rates of growth for those lambs fed cobalt salts in addition is evident. The rate of gain for those fed cobalt supplements was 0.32 pound per lamb per day for the 13 week period. This rate of gain is considered good for commercially fattened lambs. It is noted that weight gains were evident at one week following cobalt therapy. Growth of similarly treated lambs in the second experiment followed a similar pattern.

Although an anemia has not consistently been reported for cobalt de-

TABLE 1. TYPE OF ANEMIA IN COBALT-DEFICIENT LAMBS

Treatment	No. lambs	Weeks on experiment	Red cell count	Hemoglobin	Centrifuge hematocrit	Mean cell volume	Mean cell hemoglobin	Mean cell hemoglobin concentration
			(mill./cmm.)	(gms./100 ml.)	(%)	(cμ)	(rr)	(%)
First Experiment								
+Co	8	13	9.87±0.324	11.5±0.35	37.9±1.28	38.5±1.05	11.7±0.26	30.5±0.61
−Co	5	13	6.60±2.880*	7.5±0.75**	25.0±3.03**	37.9±1.99	11.5±0.45	30.5±1.20
Second Experiment								
+Co	6	12	11.23±0.451	12.0±0.48	42.5±2.02	37.9±1.00	10.8±0.26	28.4±0.61
−Co	5	12	5.43±0.669**	5.1±0.79**	19.9±2.50**	36.1±1.35	9.3±0.74	26.0±2.02

* Mean difference significant P =0.05.
** Mean difference significant P =0.01.

ficiency, it has been a constant observation in our studies. In the first experiment the anemia would be classified as mild (table 1). In the second experiment, it was more severe. Some data have been published indicating that a cobalt-deficiency anemia in lambs is microcytic and hypochromic in type (Filmer 1933). In both of the studies here reported, the anemia has been simple, that is, normocytic and normochromic as the values in table 1 clearly show.

A general summary of observations on the lambs in the first experiment is given in table 2. Those lambs fed cobalt salts had normal hemoglobin

TABLE 2. GENERAL OBSERVATIONS ON THE LAMBS IN THE FIRST EXPERIMENT

Treatment	Number of lambs	Period of treatment	Initial weight	Final weight	Final hemoglobin level	Deaths
		(wks.)	(lbs.)	(lbs.)	(gms./100 ml.)	
+Co	8	13	57±1.8	86±1.9	11.5±0.35	0
−Co	8	13	61±1.8	46±2.2**	7.5±0.76**	3

** Mean difference significant P =0.01.

levels at the end of the 13-week period and were sold as fat yearlings. Those lambs maintained on the low-cobalt ration were anemic and lost considerable weight.

Experiment 2 had as part of its design to test the effectiveness of cobalt salts variously administered, that is, feeding versus intramuscular injections. The amount of cobalt solution given at each injection was divided into three portions and injected into different sites. This was done to increase the amount of cobalt salts absorbed since some local irritation occurs at the site of injection. The effects of the various treatments—no cobalt, cobalt-fed

TABLE 3. EFFECT OF COBALT SALTS WHEN FED OR INJECTED ON WEIGHT AND HEMOGLOBIN LEVELS OF COBALT-DEFICIENT LAMBS

Treatment	Number of lambs	Period of treatment	Initial weight	Final weight	Final hemoglobin level
		(wks.)	(lbs.)	(lbs.)	(gms./100 ml.)
Co fed	7	7	60±3.7	71±2.6	11.6±0.18
Co injected	7	7	57±3.1	49±2.8**	7.1±0.64**
−Co	7	7	61±3.2	51±2.8**	7.1±0.82**

** Mean difference as compared to Co fed lambs significant P = 0.01.

and cobalt-injected are summarized in table 3. It is obvious from these data that cobalt salts injected had no effect on the course of cobalt deficiency as judged by growth and hemoglobin levels. In a previous paper (Gall, Smith, Becker, Stark and Loosli, 1949) it was shown that the rumen microflora in the cobalt-injected lambs was similar to the cobalt-deficient lambs and both unlike the cobalt-fed lambs.

In this study a preliminary attempt was made to detect a biochemical upset in cobalt deficiency. To this end blood plasma proteins, calcium, phosphorus and alkaline phosphatase activity were determined. The results of these observations are summarized in table 4.

It is noted that the cobalt-deficient lambs had slightly lower blood plasma protein levels as compared to the cobalt-fed lambs. This difference was significant (P = 0.01) only in the second experiment. The blood plasma calcium was slightly but significantly (P = 0.05) lower in the cobalt-deficient lambs. There was no significant difference between treatments so far as

TABLE 4. BLOOD PLASMA PROTEINS, CALCIUM, INORGANIC PHOSPHORUS CONCENTRATIONS AND ALKALINE PHOSPHATASE ACTIVITY OF COBALT-TREATED LAMBS

Treatment	Plasma proteins	Calcium	Phosphorus	Alkaline phosphatase activity
	%	mg./100 ml.	mg./100 ml.	mg.P/100 ml.
		First Experiment		
Cobalt fed	7.10±0.278			12.8±1.61
−cobalt	6.95±0.159			5.5±1.33**
		Second Experiment		
Cobalt fed	6.33±0.040	12.4±0.37	8.1±0.32	14.1±1.87
−cobalt	6.03±0.081**	11.0±0.28*	7.1±0.45	6.5±1.35**

* Mean difference significant P = 0.05.
** Mean difference significant P = 0.01.

blood plasma phosphorus was concerned but the plasma alkaline phosphatase activity was appreciably less ($P = 0.01$) in the cobalt-deficient as compared to the cobalt-fed lambs in the first and second experiments. Bowstead *et al.* (1942) previously reported a slight reduction in blood proteins in cobalt deficiency.

The autopsy observations were generally negative. The only observation that showed some consistency was a fatty, degenerated liver that occurred in about 57 percent of the cobalt-deficient lambs. In two cases this was accompanied by petechial hemorrhages under the liver capsule. The microscopic study of several tissues likewise was largely negative except to confirm the occurrence of fatty livers and to observe a marked hemosiderosis of the spleens. These observations have previously been recorded by Filmer (1933) and Bowstead *et al.* (1942).

Careful observations were made of the wool of all lambs. No abnormality that might be considered specific for cobalt deficiency was noted. The wool of cobalt-deficient lambs was retarded in growth and very weak fibered—a result that is very likely secondary to the general inanition.

Chemical Survey of Forages in New York

The cobalt analyses here reported are observations made on samples collected at random initially, usually from farms where troubles of unknown

TABLE 5. COBALT CONTENT OF SOME EASTERN NEW YORK HAYS

Kind of hay	No. of fields	No. of soil types	Total no. of samples	Distribution of fields according to the cobalt content of the forages			
				.01–.03 ppm	.04–.07 ppm	.08–.11 ppm	.12 ppm+
Timothy	67	12	149	20	39	8	0
Legume	10	3	15	1	3	0	6
Mixed[1]	31	—	51	10	11	6	4

[1] Hay with 10% or more of legumes.

etiology were reported in cattle. Later a more systematic study was made in St. Lawrence Country with the hope of establishing some correlation between the cobalt level of forages and soil types.

Each figure in table 5 is an average of from 1 to 3 samples taken from each field. The number of samples taken depended somewhat on the size of the field. Each sample was made up of about 50 small subsamples, thus it is felt that a good representative sample of each field was obtained. In interpreting these analyses, the reports of McNaught (1938) and Underwood and

Harvey (1938) were used. These workers agree that pasture forages containing less than 0.04 ppm of cobalt are potential deficiency areas. Cobalt values of 0.04–0.07 ppm are on the borderline of requirements. More recently Stewart *et al.* (1946) have reported data indicating that cobalt deficiency in lambs may develop on rations containing as much as 0.09 ppm. cobalt. On the basis of such reports, nearly one-third of the timothy and the mixed-legume fields sampled were classified as cobalt deficient (0.01–0.03 ppm.) and an additional 50 percent classified as borderline (0.04–0.07 ppm.). There is an indication from these data that legumes tend to run higher in cobalt than the grasses grown on the same fields. There was no consistent

TABLE 6. COBALT CONTENT OF SOME EASTERN NEW YORK TIMOTHY AND MIXED GRASS-LEGUME HAYS ACCORDING TO COUNTIES

County	Distribution of fields according to the cobalt content of the forage			
	.01–.03 ppm	.04–.07 ppm	.08–.11 ppm	.12 ppm+
Franklin	12	3	0	0
St. Lawrence	15	38	8	0
Madison	1	4	0	0
Cortland	2	3	1	1
Essex (Champlain Valley)	0	3	5	3

relationship of cobalt content of forages to soil type, partly because of the small number of samples collected from any one soil type. However, in St. Lawrence County where a more systematic study has been made, no fields on the Dunkirk, Vergennes and Parishville soils could be classified as deficient. On the other hand, in no case could all the fields on any one soil type be classified as deficient.

In table 6 there is presented the distribution of fields within each county as to the cobalt content of forage samples of timothy and mixed hays. Heavy legume mixtures were not included in this classification. The forage samples collected in St. Lawrence County are random samples whereas in the other counties the samples were selected in that they came from farms where troubles were reported in cattle. These troubles were not necessarily due to cobalt deficiency. Of the areas so far studied only eastern Essex County was found free of decidedly low-cobalt forages. It is of interest to note that the soils of this part of Essex County were developed from a very different material than were those of St. Lawrence and Franklin Counties.

Caution should be used in interpreting the cobalt analyses of forages for as McNaught (1938) and Underwood and Harvey (1938) have pointed out the correlation between cobalt content of forages and cobalt deficiency was

not perfect. Also the observation of low-cobalt forage in an area does not necessarily indicate that cattle and sheep in that area are lacking adequate cobalt for appreciable amounts of cobalt may be given in salt mixtures or in the concentrates fed. The important thing from from the standpoint of an animal is the total intake and not the intake from any one feed. Obviously unsupplemented cattle and sheep on pasture in low-cobalt areas are most likely to be deficient in this element.

Discussion

Studies here reported as well as those reported by others have not as yet established symptoms which may be regarded as specific for cobalt deficiency. Such gross symptoms as inappetence, failure of growth and anemia are too general and may easily be confused with other nutritional diseases. Also, no biochemical or microscopic lesion has yet been detected which is specifically pathognomic. This makes field diagnosis of the deficiency very difficult. In fact, the only method to date of diagnosing cobalt deficiency with some certainty is to feed cobalt salts under controlled conditions and to note a favorable response.

The complete failure of our lambs to respond to the injection of cobalt salts is in disagreement with the results of Ray *et al.* (1948) where a partial response was reported. A reconciliation of these different observations is not at the moment evident.

Since McCance and Widdowson (1944) first mentioned unpublished results showing that injected cobalt salts were ineffective, it has generally been theorized that this was evidence for a direct need by the ruminal bacteria for cobalt which was thus required by ruminants only indirectly. More direct evidence that cobalt affects the ruminal bacteria was given by Gall, Smith, Becker, Stark and Loosli (1949).

Studies by Becker *et al.* (1949) showed that cobalt-deficient lambs did not respond to the feeding or injection of vitamin B_{12}. Apparently, then, cobalt in lambs has some major function(s) other than the formation of B_{12}.

These studies showing that hay from two areas in New York State is deficient in cobalt for lambs combined with the studies of the cobalt content of New York forages leave little doubt that this state contains many cobalt-deficient areas. States which thus far have been demonstrated to have such deficiency areas are New Hampshire, Wisconsin, Michigan, Florida, North Carolina and New York. Undoubtedly as studies are extended, other states will be added to this list.

Summary and Conclusions

Feeding trials with lambs have clearly shown that New York State contains areas that are deficient in cobalt.

Lambs fed a low-cobalt ration developed a deficiency of this element in 4–7 months characterized by loss of appetite, loss of weight, a simple anemia and death. Cobalt salts given to cobalt-deficient lambs by mouth resulted in a return of appetite within about a week. This was quickly followed by increased weight gains. Hemoglobin levels started to rise sometime later, about six weeks.

Cobalt salts injected into cobalt-deficient lambs were completely ineffective in altering the course of the deficiency.

As compared to cobalt-fed lambs, cobalt-deficient lambs showed a slightly lower concentration of blood plasma proteins; a slightly lower blood plasma calcium concentration; a lower blood plasma alkaline phosphatase activity and about the same blood plasma inorganic phosphorus concentration.

Autopsy and microscopic tissue studies showed that cobalt-deficient lambs had fatty degeneration of the livers and a marked hemosiderosis of the spleens.

A chemical survey of forage plants in the State has given some estimate of the extent of cobalt-deficient areas.

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PROTEIN AS AN INDICATOR OF PASTURE FORAGE DIGESTIBILITY[1]

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THE best chemical technique so far developed for determining the digestibility of forages by grazing animals includes the analysis of forage and feces to determine their lignin content. While this method gives fairly satisfactory results, the lignin analysis is expensive in terms of time and technical labor and is subject occasionally to unaccountable variations. The used of a simpler chemical technique might decrease the expense and variability inherent in the lignin analysis. It was with this view in mind that the following study of the use of protein as an indicator of pasture forage digestibility was undertaken.

Several approaches have been made previously to the problem of using forage or feces protein, or a combination of these, in the estimation of forage digestibility. Gallup *et al.* (1948) proposed that a constant relationship exists between the amount of fecal nitrogen and the dry matter intake, regardless of the amount of protein in the forage. Data published by Forbes (1949) and by Blaxter and Mitchell (1948) have shown this proposal to be inaccurate. Lancaster (1949) proposed a division of forages into two classes, based on their protein contents; for forages with less than fifteen percent protein on the dry basis he found that $0.67 \pm .120$ grams of nitrogen appeared in the feces for every 100 grams of organic matter intake, and that for forages with fifteen or more percent protein the corresponding figure was $0.80 \pm .081$. This appears to be an improvement over the method of Gallup since it recognizes that the forage protein level may have some effect on the amount of fecal protein per unit of dry matter intake. However, it is not a perfect solution to the problem, since there is no reason to expect a sharp break in the relationship between fecal nitrogen and organic matter intake, as is indicated by the method of Lancaster.

Mitchell (1942) found the relationship between forage protein content and apparent digestibility of protein to be of an exponential nature and expressed by the formula,

$$Y = 42.64\ (P-5)^{.2148}, \qquad (1)$$

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

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in which Y is the apparent digestibility of protein in forage of protein content P. It appeared that use of such an equation might be made in calculating the digestibility and the intake of forages of known protein content. In this paper the results of the author's studies along this line are presented.

Experimental

Data from our 1947 and 1948 grazing and digestion trials with both sheep and steers have been used in making the following calculations (Forbes and Garrigus, 1948 a, b). The data of Morrison, as interpreted by Mitchell (1942), and the data of Schneider (1947) have been used for purposes of comparison. The forages considered include both grasses and legumes. The protein content of the dry matter of the forages ranged from 5% to 33%.

If the percentage of an indigestible constituent be measured in the dry matter of feed and of feces, the ratio of these percentages will indicate the fraction of original feed dry matter that remains in the feces, since the removal of digestible matter will result in a correspondingly increased concentration of indigestible matter. Expression of the above ratio as a percentage and subtraction from 100 will give the dry matter digestion coefficient. This may be expressed by the following equation:

$$D = 100 - 100 \frac{\% \text{ indigestible matter in dry feed}}{\% \text{ indigestible matter in dry feces}}. \qquad (2)$$

The percentage of indigestible protein in the dry matter of the feed may be calculated by multiplying the protein percentage of the feed dry matter by 1 minus the coefficient of protein digestibility; the latter figure may be obtained from the equation, $Y = aX^b$, in which "a" and "b" are constants and X is five less than the protein content of the forage dry matter. The assumption is made that the total protein in the feces represents indigestible protein, and this is expressed as a percentage of the fecal dry matter. A convenient form of equation 2 then becomes:

$$D = 100 - \frac{\% \text{ protein in feed}}{\% \text{ protein in feces}} (100 - aX^b). \qquad (3)$$

We found, using the values for "a" and "b" obtained by Mitchell, that the average digestibility of dry matter in our steer data was not different from that calculated by the conventional or by the lignin ratio methods. In the case of the sheep data, however, a discrepancy was found in the comparison of the averages obtained by lignin ratio and by "protein digestibility"

methods as applied to the digestion, or "inside" trials. The protein digestibility method gave average results similar to those obtained by the conventional method in these trials. In spite of the general agreement of average results, the slope of the regression lines relating dry matter digestibility to lignin content of the forage was less when the protein digestibility method was used than when either of the other two methods was used. This led to an investigation of the applicability to our data of the constants in Mitchell's equation.

TABLE 1. CONSTANTS FOR EQUATION $Y = aX^b$, SHOWING RELATIONSHIP BETWEEN PROTEIN DIGESTIBILITY (Y) AND PROTEIN CONTENT ($X+5$) OF THE DRY MATTER OF FORAGE

Source of data	No. of feed classes	a	b	Standard error of equation	Correlation of X and Y	Standard error of b
				%		
Kentucky—Cattle and Sheep	62	40.96	.2263			
Schneider—Cattle and Sheep	501	40.31	.2181			
Mitchell—Cattle and Sheep	163	42.64	.2148			
Kentucky—Cattle	19	35.40	.2874	+4.7 −4.6	.90	.0333
Schneider—Cattle	118	35.31	.2717	+2.8 −2.7	.99	.0030
Kentucky—Sheep	43	42.73	.2099	+7.5 −7.0	.81	.0239
Schneider—Sheep	383	42.13	.2003	+6.5 −6.1	.90	.0052

Using nineteen grazing and digestion trials (each employing at least three animals) with steers and forty-three with sheep, we fitted our data for sheep and steers separately, and together, to the equation, $Y = aX^b$ (table 1). The data involving roughages from Schneider's (1947) compilation were selected and fitted to the same general equation.

It is apparent from a study of table 1 that calculations from our data lead to results similar to those obtained from other sources also shown in figure 1 An unexpected finding was that the exponent of the equation was consistently greater for cattle than for sheep. The difference between exponents is

highly significant in accordance with Student's "*t*" test only in the comparison of Schneider's sheep and cattle data. The comparison of Kentucky sheep and cattle data shows a significant difference at the 5% level. Although all of the correlation coefficients are highly significant, that for our sheep data is the lowest of the group. This may be ascribed to the fact that lignin is a more important determinant of total digestibility than is protein, and in our sheep trials there was a lower degree of correlation (negative) between forage lignin and forage protein than in our steer trials (Forbes and Garrigus, 1948).

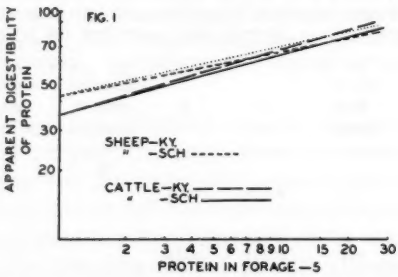


Figure 1. Plotted on logarithmic scale. Showing relationship between protein level in dry matter of forage and apparent digestibility of protein by cattle and by sheep. Data from Forbes and Garrigus (1948 a, b) and from Schneider (1947).

The next step was to select the appropriate values for "*a*" and "*b*", and then, using equation 3, calculate the dry matter digestibility. Regression equations relating dry matter digestibility to lignin content of the forage were then calculated for the several related groups of experimental animals, and resultant equations were compared with those previously calculated from the data obtained by the lignin ratio or conventional methods (table 2).

Within-group comparisons of the slopes of the above regressions indicate, according to Student's "*t*" test, that only in the case of the outside lamb data is there a significant difference. Within group comparisons of the average digestibility of dry matter calculated in the various ways indicate that statistical differences at the 1% level occur only in the comparison of inside lamb data calculated by the lignin ratio to that calculated by the protein digestibility method, and to that calculated by the conventional method.

A consideration of the above data indicates that the calculation of dry matter digestibility from predicted protein digestibilities fails to attain the expected accuracy in all cases. With the exception of the grazing steer data, low lignin forages actually are more digestible than the results of such a calculation indicate (figures 2–5). In the instance of the outside lambs, the

TABLE 2. REGRESSIONS OF DRY MATTER DIGESTIBILITY (Y) ON LIGNIN CONTENT (X) OF THE DRY MATTER OF FORAGE

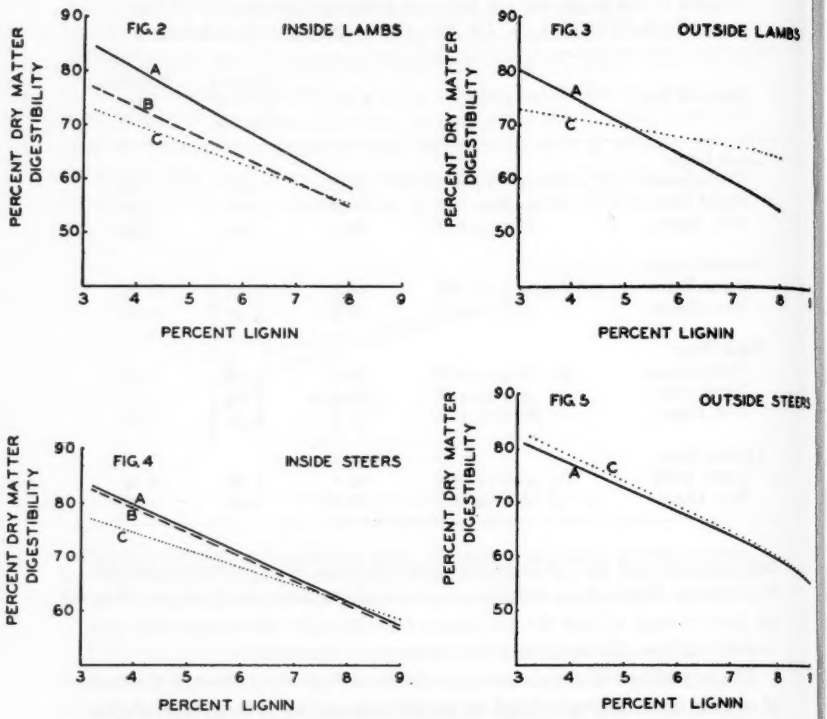
Source of data	$Y=a+bX$	Ave. Y	Standard error of equation	Standard error of b
Inside Lambs				
Conventional	$Y=92.5-4.72X$	63.7	4.30	1.45
Lignin Ratio	$102.8-4.58X$	68.8	3.78	1.28
Prot. Digest.	$84.5-3.62X$	62.4	5.24	1.77
Outside Lambs				
Lignin Ratio	$Y=95.7-5.18X$	68.8	3.56	0.46
Prot. Digest.	$78.8-1.92X$	68.8	4.47	0.20
Inside Steers				
Conventional	$Y=96.4-4.44X$	64.9	3.08	0.70
Lignin Ratio	$97.4-4.44X$	65.9	3.08	0.70
Prot. Digest.	$87.6-3.22X$	64.8	2.84	0.64
Outside Steers				
Lignin Ratio	$Y=96.0-4.49X$	69.1	1.58	0.29
Prot. Digest.	$97.6-4.66X$	70.6	4.82	0.88

high lignin forages are less digestible than indicated by the protein digestibility methods. While these differences are not all statistically significant, they are great enough to limit the usefulness of the concept; this is especially true if small numbers of animals and periods are to be compared.

The regressions of dry matter digestibility on protein content in the case of outside lamb data calculated by lignin ratio and by protein digestibility methods are not significantly different (table 3). Comparison of the slopes of these lines by the "t" test indicates no significant difference. These data indicate that the lignin content is associated with digestibility in such a way that the "plus" errors in calculations by the protein digestibility method are grouped with the high lignin values. In most of our data (figures 2, 3, 4, 5) the negative errors exceed the positive ones in frequency and magnitude.

TABLE 3. REGRESSION OF DRY MATTER DIGESTIBILITY (Y) ON PROTEIN CONTENT (X) OF THE DRY MATTER OF FORAGE CONSUMED BY GRAZING LAMBS

Method	$Y=a+bX$	Standard error of equation	Standard error of b
Lignin Ratio	$Y=53.3+.847X$	8.0	0.188
Prot. Digest.	$Y=55.9+.707X$	5.2	0.096



Figures 2, 3, 4, 5. Showing relationship between lignin content of dry matter of forage and digestibility of dry matter by cattle and sheep as calculated by lignin-ratio (A), conventional (B) and protein-ratio (C) methods.

Summary

A method of calculating dry matter digestibility of forages from the protein content and predicted protein digestibility is presented. The data obtained are compared with data obtained by conventional and by lignin-ratio methods of determining digestibility. The average digestibilities calculated in the various ways from data of lamb and steer trials are, in general, similar, but the slopes of the regressions of dry matter digestibility on lignin content are generally less when data are obtained by the "protein digestibility" method. This difference in slopes is statistically significant only in the case of the lamb trials. It would appear from the data reported in this paper that the method may be used with a satisfactory degree of accuracy for the determination of digestibility of dry matter by grazing steers.

A difference in digestive capacities of cattle and sheep has been demonstrated; sheep are apparently more efficient digesters of protein in low-protein forage than are cattle. The difference disappears above about 15% protein in the dry matter of the forage.

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EFFECTS OF DIETHYLSTILBESTROL DIPROPIONATE UPON POSTPARTUM CHANGES IN THE COW[1]

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TREATMENT of breeding cows with stilbestrol has been considered appropriate in cases of retained after-birth, pyometra, mummified fetus and anestrus. Two recognized physiological actions of estrogen appear to underlie its use in these different cases, namely, its stimulatory action upon the musculature (Frank *et al.*, 1925) and mucosa (Allen, and Doisy, 1923) of the genital tract and its presumed ability to initiate the luteinizing action of the anterior pituitary gland (Hohlweg, 1934; Lane, 1934).

An increase in the contractility of the uterine muscle together with a relaxation of the cervix is believed to put the genital organs in better mechanical condition to rid themselves of non-physiologic contents such as pus and dead fetuses and membranes. The stimulating action upon the genital epithelium is presumed to bring about faster reparative changes in damaged mucosa and also to cause increased or modified secretory activity which in itself might produce favorable changes in the genital flora (probably in the vaginal or cervical regions). In addition, the supposed increase in quantity of secretions is thought to act as a beneficial lavage or flush of the genital tract.

The release of luteinizing hormone by the anterior pituitary gland when under the influence of estrogen has seemed a logical reason for the use of stilbestrol in anestrual animals to initiate luteinizing activity on the part of the animal's own gland to bring about pre-ovulatory swelling of the follicle and ovulation. If this treatment in itself does not lead immediately to fertility it is further presumed that some delayed action of the treatment will bring about a normal heat period approximately the interval of one estrual cycle later.

The imposing array of clinical conditions for which stilbestrol has been proposed together with the low cost and wide spread availability of the chemical has led to its use becoming widely familiar to dairymen. The expense has become so little that in many instances dairymen are presuming it worth-while to treat all cows just after calving whether or not any abnormality appears. They are doing this in the belief that there will be an improvement in the breeding efficiency of the herd as a whole. The present

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Paper from the Department of Genetics (No. 413) and from the Department of Veterinary Science.

controlled experimental study was made to determine whether an effect, beneficial or deleterious, could be detected from such a treatment.

Material and Methods

Holstein cows[2] of various ages were used in this study—60 animals in all. The cows were divided into pairs on the basis of age (parity) and season of calving. One member of each pair was injected intramuscularly within nine hours after calving with five cc. of Abbott's Stilronate (kindly furnished by Abbott Laboratories) containing a total of 20 milligrams diethylstilbestrol dipropionate. The other member served as the control.

There were only three exceptions to an exact pairing on the basis of parity and in those instances a seventh calving cow was paired with a fifth, a fourth with a seventh, and a second with a third. The calving dates ranged from April 12, 1944 to January 5, 1945. The interval between calving dates for the two members of a pair ranged from one to 73 days with the members of 24 out of the 30 pairs calving no more than one month apart. The pairings were made and then designation was made at random before calving as to which cow was to be treated.

In the case of a retained placenta the animals were treated with four $\frac{1}{8}$ ounce capsules of silver oxide ointment (Squibb) introduced into the uterus and this was followed when necessary by manual removal of the placenta a few days later. Both experimental and control animals alike were treated in this way. Only five cases of retained placenta occurred—four of these by chance were in the treated group and one in the control.

Data were gathered on the length of the intervals following parturition until (1) completion of involution of the uterus; (2) ovulation, and (3) estrus.

The observations on rate of uterine involution were made by manual palpation of the genital organs, per rectum. Two different examiners made the observations, both members of a pair of cows were checked by the same examiner, not knowing at the time which cow was treated and which was the control. These examination periods were scheduled at 14 day intervals for the whole herd with the first examination occurring between eight and 21 days postpartum for the individual cow depending upon the date of calving relative to the date of the examination day. Further examinations were made until the uterus had involuted and the cow had been in heat. One examiner, (L. E. C.) studied 16 pairs and found an average interval until the uterus was involuted of 27.8 days. The other examiner (W. W.) studied 14 pairs for an average of 31.3 days. The difference between these two values is

[2] Cattle for this study were made available by the Wisconsin Department of Public Welfare through the courtesy of Mr. W. W. Kinyon. The authors are indebted to Mr. T. C. Webster, in charge of the dairy herd at Winnebago State Hospital, for observations of estrus on these cows and for injection of them with Stilronate.

TABLE 1. EFFECT OF TREATMENT WITH DIETHYLSTILBESTROL DIPROPIONATE UPON POSTPARTUM INTERVALS

Calving interval	Number pairs	Interval in days from calving to:								
		Involution of uterus			Ovulation			Heat		
		Control	Treated*	Both	Control	Treated*	Both	Control	Treated*	Both
1st	12	25.2	27.8	26.5	29.4	31.8	30.2	63.9	53.0	58.5
2nd and 3rd	10	28.9	26.1	27.5	27.0	32.4	29.7	39.4	51.3	45.4
4th and up	8	31.2	41.2	36.2	51.8	44.2	48.0	91.4	72.6	82.0
All	30	28.0	30.8	29.4	34.6	35.3	35.0	63.1	57.7	60.4

* 20 mg. diethylstilbestrol dipropionate (5 cc. Abbott's Stilronate) were injected intramuscularly within 9 hour after calving.

not statistically significant. The criteria used when judging complete involution of the uterus were (1) return of the organs to a pelvic or near-pelvic position; (2) reduction of the size of the two horns until they were essentially equal and showed no further reduction in size; (3) development of "normal" consistency and tone of the non-pregnant uterus. The cows were further examined between 4 and 17 days after artificial insemination to determine

TABLE 2. ANALYSIS OF THE VARIANCE IN POSTPARTUM INTERVALS

Source of variation	D/F	Mean square for interval from calving to:		
		Uterine involution	Ovulation	Heat
Treatment (Tr.)	1	115	9	437
Number of calving intervals (C.I.)	2	516	1862*	6042**
Tr.×C.I.	2	182	198	1196
Cows within C.I. and Tr.	54	214	579	1014
Pairs within C.I.	(27)	(201)	(480)	(783)
Pairs within C.I.×Tr.	(27)	(228)	(677)	(1245)

* P=0.05.
** P=0.01.

the location of the corpus luteum and also between 34 and 47 days after breeding to determine pregnancy if there had been no ensuing estrus. The method of Wisnicky and Casida, 1948, was used in this study.

Heat periods were checked by observing for cows standing to be mounted by other cows when they were turned from the barn into the paddock each day. Cows showing restlessness, bawling in the barn, holding up of milk or a flow of fluid mucus from the vulva were observed particularly when turned into the paddock. The cows were milked three times daily at approximately eight hour intervals.

The time at which involution of the uterus or quiet ovulation occurred was for the purposes of study judged to be half-way between the last examination when it had not occurred and the first examination when it had occurred.

Experimental Results

It was anticipated at the beginning of the experiment that if there was a beneficial effect from treatment the most likely place for it to be expressed would be upon the interval from calving until involution of the uterus. The average interval for all cows, treated and controls, was found to be 29.4 days (table 1). This is in fair agreement with the finding of 26.2 days by Casida and Venzke, 1936. Analysis of the data showed there to be no significant effect of treatment (table 2). Further, there were no significant differences among the cows that had different numbers of calving intervals; thus, age did not appear to have a definite effect on the rate of uterine involution. Also, there was no evidence that different calving-interval-groups reacted in a different way to treatment.

The interval from calving until the first ovulation was found to average 35.0 days (table 1). Again, the length of this interval was not significantly affected by treatment (table 2). Significant differences were found however, between the different calving-interval-groups. Those in the first calving interval averaged 30.2, those in the second and third 29.7, whereas those in the fourth and later averaged 48.0 days. Although the older cows showed a somewhat longer interval before full ovarian activity was resumed, there was no evidence that the different groups reacted to treatment in a significantly different manner.

The average interval from calving until the first succeeding heat period was 60.4 days (table 1). This is longer by 25 days than the length of the interval from calving until ovulation. "Quiet" ovulations had of course been recognized before this study. The large proportion of cows, however, which showed ovulations before they showed estrual behavior, 68% was not anticipated.

Again there was no significant effect of treatment on the average interval from calving to the first heat period. Also, just as different calving interval groups varied in the interval from calving to the first ovulation, again they varied in the interval from calving to the first heat. The first calving-interval group average 58.5 days, the second and third 45.4 and the group including those of the fourth and later calving intervals average 82 days. Although the different aged cows had different average intervals until the first heat period following calving, there was no statistically significant evidence that one group gave any more of a response to estrogen than another.

A possible harmful effect of treatment might be the development of cystic ovaries. Such a condition has been reported frequently following the use of stilbestrol. A total of five cases occurred in these animals by the time of first estrus; three of these cases were in the treated animals and two in the controls. Thus no significant difference in the occurrence of the cystic condition was found as a result of treatment.

The majority of the cows on this experiment were bred (artificially inseminated) during the progress of the study. Diagnosable pregnancies at 34 to 48 days after first insemination were found in 43% of the 28 control cows which were bred and in 38% of treated cows. Thus there was no evidence that treatment improved the breeding efficiency of these cows despite the fact that the performance of the control cows would ordinarily be considered as fairly low.

Summary

The effects of diethylstilbestrol dipropionate upon postpartum changes in the genital organs and upon subsequent breeding efficiency has been studied in 60 purebred Holstein cows. Injection of 20 mg. of the hormone within 9 hours after calving failed to produce a demonstrable effect on the intervals from calving until (1) involution of the uterus (2) first succeeding ovulation, or (3) first succeeding estrus. No effect of treatment was shown upon the occurrence of cystic ovaries or the percentage of cows conceiving upon first service. Ovulation, on the average over all cows, occurred 25.4 days earlier than the first postpartum estrus; 68 percent of the cows showed at least one "quiet" ovulation before the first estrus. Older cows (fourth and later calving intervals) tended to show longer intervals from parturition to estrus and to ovulation than did younger cows.

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COMPARATIVE VALUE OF MIXED HAY AND CORN SILAGE FOR WINTERING BEEF COWS

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MIXED legume-timothy hay and corn silage are commonly fed in wintering rations for dry, mature beef cows, especially in the Northeastern and North Central States. These roughages have been fed successfully either as the sole roughage or in various combinations. In trials reported by Leinbach (1944, 1945), brood cows fed liberally on corn silage as the only roughage but receiving a protein supplement and ground limestone made greater gains and produced heavier calves than similar cows fed liberally on mixed clover-light timothy hay plus a mineral supplement.

The objects of the series of four trials herein reported were the following: (1) to determine the relative feeding value of corn silage in comparison with that of mixed legume-timothy hay for wintering dry, mature beef cows; (2) to determine the amount of mixed hay or mixed hay and corn silage required for wintering such cows in a good state of nutrition; and (3) to determine the differences, if any, in birth weight of calves produced by cows fed mixed hay only and those fed mixed hay and corn silage.

Experimental

Animals Used

The cows used in each of the four trials were from the purebred breeding herds maintained at Cornell University. Both Aberdeen-Angus and Hereford cows were used in each trial. Cows were selected that had nursed calves during the preceding summer, were due to calve the following spring, and were mature or at least had produced two calves prior to the trial. Minor exceptions occurred due to the relatively few cows available and to the fact that pasture breeding was practiced in these herds.

These cattle had in all cases grazed on good, improved bluegrass-white clover pastures the summer preceding wintering. The calves were creep-fed. As indicated later, the cows were in good condition when they came off pasture and the calves were weaned. In each trial the cows were removed from pasture to winter quarters during the last week of October or the first week of November, averaging about November 1. In most cases the calves had been weaned one or two weeks prior to this time. Each trial was started

* The authors wish to acknowledge the aid of James Briggs, Alex Martins, Floyd Blaser, and Wayne Thorndyke in the conduct of the trials and of Kenneth Tillapaugh and William Slaight, who fed the experimental cattle.

after the silos had been opened for winter feeding, with the exception of Trial I which was delayed until December 12, because the wintering shed for the cows was not completed until this date. The average starting date for the four trials was November 25.

At the start of each trial, two lots of 7 cows were selected. These lots were comparable on the basis of weight, degree of fleshing, age, breed, and calving records.

Feeding Plans

The feeding plans for each of the two lots of cows were as follows:

Lot I. These cows were hand-fed mixed hay once each day and had free access to a mineral supplement (ground limestone, steamed bonemeal or dicalcium phosphate, and salt) and to block salt. The mixed hay was fed liberally but at no time in such amounts that hay accumulated in the rack. In one trial (Trial II) essentially pure timothy hay and alfalfa hay were fed on alternate days to provide the mixed hay.

Lot II. These cows were fed one-half the amount of hay fed daily to Lot 1 and sufficient corn silage, in addition, to permit this group of cows to make the same gains in weight as the cows in Lot I.

All cows were weighed at regular intervals, usually every 14 days, and feeds were adjusted as needed to equalize gains between lots. All roughages were fed in an inside feeding trough and hay rack, with each day's allowance given in the mornings. The cattle were fed in an open shed with outside exercise lots. The shed was kept well-bedded with straw, but no appreciable amount of straw was consumed by the cattle.

The cows were pasture bred to start calving soon after March 25. At the latest date possible prior to calving, the trial was terminated so far as feed records and weight records were concerned. The average ending date for the four trials was March 29. However, the cows were kept in their respective lots and birth weights of the calves were taken until the start of the pasture season. The same feeds for each lot were continued and in the same amounts, except that 3 lbs. of a mixed concentrate feed were fed per head per day to the cows after several calves had been born in each lot.

Feeds Used

The mixed hay varied somewhat from year to year but in general it was U. S. No. 2 alfalfa or clover, timothy mixed hay. The hay varied from 30 to 50 percent legume, with the remainder largely timothy and other grasses. The corn silage was produced locally and ensiled in the dough stage. The corn was judged fair to good in grain content. The composition of the feeds is shown in table 1.

TABLE 1. COMPOSITION OF FEEDS USED (PERCENT)

Year	Feed	Legume	Dry matter	Protein	Fat	Fiber	Nitrogen-free extract	Ash
Trial I								
1944–45	Mixed hay	31	92.5	7.1	2.1	37.3	41.5	4.5
	Corn silage	—	27.0	2.0	0.4	6.8	16.2	1.6
Trial II								
1945–46	Mixed hay	50	88.0	7.9	1.2	36.0	38.0	5.5
	Corn silage	—	32.0	2.3	0.5	9.4	17.6	2.2
Trial III								
1946–47	Mixed hay	50	86.4	10.4	1.8	31.0	38.2	5.0
	Corn silage	—	19.5	1.6	0.5	6.0	10.4	1.0
Trial IV								
1947–48	Mixed hay	30	89.9	7.3	1.9	36.9	39.1	4.7
	Corn silage	—	26.2	2.9	0.8	9.7	11.5	1.3

Results

The results of each of the four trials and an average for the trials are shown in Table 2.

As mentioned previously, Lot I was wintered on a liberal feeding of mixed hay. The average amounts fed daily for each trial varied from 21.6 lbs. to 23.2 lbs., averaging 22.5 lbs. for the four trials. On this ration, the cows gained from 0.20 lb. to 0.48 lb. per head daily per trial, averaging 0.34 lb. for all trials. The cows remained thrifty at all times, and on the average ended the trials in about the same condition as at the start, or in good condi,

TABLE 2. RESULTS OF FEEDING TRIALS

Item	Mixed hay alone					Mixed hay and corn silage				
	Lot I					Lot II				
Trial No.	I	II	III	IV	Av.[1]	I	II	III	IV	Av.[1]
No. cows in lot	7	7	7	6[2]	7	7	7	7	7	7
No. days on trial	98	133	146	121	124	98	133	146	121	124
Av. initial wt., lbs.	1156	1217	1189	1212	1193	1162	1214	1195	1219	1198
Av. da. gain/cow, lbs.	.31	.20	.48	.35	.34	.35	.21	.47	.36	.35
Av. daily feed consumed per cow										
Mixed hay, lbs.	22.6	21.6	22.8	23.2	22.5	11.2	10.8	11.4	11.6	11.2
Corn silage, lbs.						23.4	25.9	28.7	28.4	26.9
Av. no. lbs. silage to replace 1 lb. mixed hay						2.1	2.4	2.5	2.5	2.4
Av. grade of cows at start	G	G	G	G−	G	G	G	G	G	G
Av. grade of cows at end	M+	G	G+	G−	G	G	G	G	G	G
Av. birth wts. of calves, lbs.	69	72	58	64	66	68	65	64	62	64

[1] The averages for the four trials are based on records for each cow and not on averages by trials.

[2] One cow in Lot I (Trial IV) proved not to be with calf and was removed from experiment.

tion. The cows in Lot II were fed one-half as much hay as those in Lot I, or an average of 11.2 lbs. per head daily for the four trials. Then, in addition, they were fed corn silage in amounts believed proper to permit them to gain at the same rate as the cows fed mixed hay only. The average amount of corn silage fed per cow daily ranged from 23.4 lbs. to 28.7 lbs. for the four trials, averaging 26.9 lbs.

The gains made by each lot averaged very nearly the same. The greatest difference between lots occurred in the first trial in which the cows in Lot II (mixed hay and corn silage) made an average of 5 lbs. greater gain for the entire feeding period. The average daily gain for the four trials was c 34 lb. per head daily for Lot I, compared with a gain of 0.35 lb. for Lot II.

The cows fed mixed hay and corn silage (Lot II) averaged about the same in condition at the end of the trials as Lot I, but if there was a difference, Lot II had the advantage. In fact, after the trials were in progress for a month to six weeks, the cows fed corn silage appeared to have more "bloom" or sleeker haircoats than the other lot, even though they were not gaining more in weight. Also, the cows fed corn silage appeared to have better appetites, and at all times would have consumed more corn silage had it been offered.

Assuming equally satisfactory nutrition for each lot, it is possible to calculate the feeding value of corn silage in terms of mixed hay. As shown in table 2, the amount of corn silage required to replace a pound of mixed hay averaged 2.1 lbs., 2.4 lbs., 2.5 lbs., and 2.5 lbs. for each trial, respectively, or an average of 2.4 lbs. for the four trials. Thus, these trials indicate that for wintering dry, mature beef cows, corn silage fair to good in grain content harvested in the dough stage and containing an average of approximately 26 percent dry matter, has a feeding value per pound of 42 percent that of U. S. No. 2 mixed legume-timothy hay ($\frac{1}{3}$ to $\frac{1}{2}$ legume), when sufficient mixed hay is included in the ration to supply adequate protein.

In these trials, 1.85 lbs. of mixed hay were fed daily per 100 lbs. live weight to Lot I and 0.92 lb. mixed hay and 2.21 lbs. corn silage per 100 lbs. live weight to Lot II. These amounts of feed essentially maintained cows in good condition that were in good condition in the fall. Most purebred breeders would consider that these cows had been wintered satisfactorily, while a commercial cattle producer would consider them as fat. Undoubtedly cows which are thin in the fall would improve in condition on this rate of feeding. Also, the cows used in these trials would no doubt have produced and raised satisfactory calves had they been fed somewhat less liberally.

Birth weights were taken on all calves born before the start of the pasture season. Not all cows had calved by this time, but weights were not taken on calves born on pasture. The 18 calves weighed from cows fed mixed hay (Lot I) averaged 66 lbs. at birth, compared with an average birth weight of

64 lbs. for 17 calves born from cows in Lot II. There was no significant difference in the birth weights of calves between the two lots. The difference in average birth weights that appears in a particular trial is due largely to the relative proportion of Hereford and Angus calves that had been born in each lot, as the Hereford calves averaged heavier at birth than the Angus calves. Weights were also taken of all calves at weaning time, and no apparent differences were found.

It was of interest to note how the cows responded throughout a full year to the feeding and management program followed and as described previously. Although the experiment was not designed especially to study this problem, such data were available, as some cows were used in more than one of the first three trials. There were 15 comparisons in which cows were weighed and graded for condition in the fall after they had nursed a calf during the preceding summer; had been wintered in the experiment and nursed a calf the second summer; and then had been weighed and graded the following fall for the start of a second trial. A summary of these data show that the cows weighed an average of 1,251 lbs. at the start of the wintering period; 1,293 lbs. when weighed in late March prior to calving in the spring; and 1,246 lbs. at the start of a second trial and after nursing a calf during the summer. The cows averaged good in condition at each of the three weighings. Thus, these data indicate that the feeding and management practices followed maintained the cows fully in both weight and in good fleshing for a purebred herd during a complete year of production.

Summary

A series of 4 trials have been conducted with dry, mature beef cows to study the relative feeding value of U. S. No. 2 mixed legume and timothy hay (30 to 50 percent legume) with corn silage fair to good in grain content and harvested in the dough stage. In these trials 2.4 lbs. of the corn silage were equal in feeding value to 1.0 lb. of the mixed hay or the corn silage was worth 42 percent as much on a weight basis as the mixed hay when sufficient protein was furnished in the ration.

Cows in good condition at the start maintained this condition throughout the trial when fed daily either 1.85 lbs. mixed hay per 100 lbs. live weight, or 0.92 lb. mixed hay and 2.21 lbs. corn silage per 100 lbs. live weight. There was no significant difference in the birth weights of calves between lots.

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MEMORIAL

FREDERICK W. CHRISTENSEN

Frederick W. Christensen, 70, who retired as professor of animal nutrition at the North Dakota Agriculture College, Fargo, North Dakota in 1947 after 31 years of service, died July 31, 1949 at Osage City, Kansas, where he had resided since his retirement.

Born at Randolph, Kansas, January 22, 1879, Mr. Christensen was graduated from Kansas State College in 1900. He did graduate work at Pennsylvania State College and Yale University and received his Master's Degree from the latter institution. He was an animal nutrition expert for the United States Department of Agriculture for six years and served in the experiment stations of Pennsylvania State and New Mexico State colleges before he came to the North Dakota Agricultural College in 1916.

He was elected as president of the North Dakota Academy of Science in 1941 and in the same year served as president of the North Dakota-Kansas State Alumni Association. He was a member of the State Alumni Association. He was a member of the American Chemical Society, American Association for the Advancement of Science, American Society of Animal Production, and Phi Kappa Phi. He was widely known for his work with emergency drought rations for beef cattle and work horses and the use of leafy spurge, sweet clover silage, Russian thistle and beet tops in animal diets.

Mr. Christensen married Alma Johnson at Osage City September 1, 1909. In addition to his wife, he is survived by three sons, Charles Frederick, Arthur George, and Paul Edward

NEWS AND NOTES

The forty-second annual meeting of the American Society of Animal Production will be held at the Hotel Sherman in Chicago, Illinois, on November 24 and 25, 1950, with a dinner for the honored guest on November 26. Members planning to present papers are reminded that titles must be in the hands of the secretary, Dr. H. M. Briggs, Animal Husbandry Department, Oklahoma A. and M. College, Stillwater, Oklahoma, by July 20, following which abstracts must be filed with him by August 15.

Nominations for the 1950 Morrison award must be in the hands of the secretary, Dr. H. M. Briggs, by June 15. The rules governing the selection of the winner of this award have been published in the JOURNAL OF ANIMAL SCIENCE, Volume 5, pages 236–237, and an amendment to the rules in Volume 7, page 257.

Nominations for the 1950 American Feed Manufacturers Association award must be in the hands of the secretary, Dr. H. M. Briggs, by June 15. The rules governing the selection of the winner of this award were published in the JOURNAL OF ANIMAL SCIENCE, Volume 7, pages 257–258.

Erratum—the name of the 1949 honor guest should have been reported as John Arthur Hill in the Proceedings of the Society meetings reported in Volume 9, pages 128 and 129.

The Committee on Ralston-Purina Fellowship Awards has selected the winners for the school year of 1950–1951. The principals named for Animal Husbandry were Elbert J. Turman of Arkansas, and Conrad J. Kercher of Montana. The institutions at which these men preferred to work were Purdue and Wisconsin respectively. Raymond F. Sewell of Florida was named as first alternate and Vaughn C. Speer of Iowa as second alternate. The Dairy Husbandry principals were James T. Miles of Tennessee and Richard G. Warner of Maryland. These men preferred to continue their work at Wisconsin and Cornell respectively. Richard Francis Davis of New Hampshire was named as first alternate, and Harry R. Conrad of Kentucky as second alternate. The principals for Poultry Husbandry were James R. Jowsey of Saskatchewan, and Rollin H. Thayer of Oklahoma. MacDonald College and the State College of Washington were the institutions at which these men preferred to continue their work. Edward C. Naber of Wisconsin, and W. A. Glista of Illinois, were named as first and second alternates respectively. The nature of these awards and some of the rules governing them were published in the JOURNAL OF ANIMAL SCIENCE, 1949, Volume 8, page 165.

H. T. Converse retired as of February 28, 1950, after 34 years of service in (progressively) the Dairy Division, the Bureau of Dairying, and the Bureau of Dairy Industry, U.S.D.A.

A meeting of Livestock Breeding under Tropical and Sub-Tropical Conditions was held in Lucknow, India, under the auspices of the Food and Agriculture Organization of the United Nations, from 10–22 February 1950. Representatives of the following countries were in attendance: Australia, Belgium, Burma, Ceylon, Egypt, France, India, Indonesia, Iran, Italy, Pakistan, Thailand, and the United Kingdom. Ralph W. Phillips served as Secretary-General of the meeting, and N. R. Joshi, also of the FAO staff, and Douglas H. K. Lee of Johns Hopkins University served as technical secretaries.

H. R. Varney resigned the positions of director of extension and dean and director of agriculture at the New Mexico College of Agriculture, and on March 1 became dean of agriculture and director of the experiment station, West Virginia University.

J. A. Hill of the University of Wyoming has been made vice president of the institution, an active position, and will also be dean emeritus of the agriculture college and director emeritus of the experiment station.

Willard M. Fifield was made director of the Florida Agricultural Experiment Station, effective March 1, to succeed Harold Mowery, retired.

C. J. Sierk of the animal husbandry staff, University of Minnesota, has accepted the position of assistant coordinator for the Western region in the cooperative cattle breeding program of the Bureau of Animal Industry, with headquarters at Denver, Colorado.

J. C. Grimes has relinquished administrative responsibilities in the Animal Husbandry Department of Alabama Polytechnic Institute, but will continue as a member of the staff. W. D. Salmon succeeded Mr. Grimes as head of the department, effective February 1, 1950.

C. A. Cary, who has been head of the division of nutrition and physiology of the U. S. Bureau of Dairy Industry since 1940, recently retired and was succeeded by L. A. Moore, his former assistant.

E. J. Warwick, of the Purdue University animal husbandry staff has accepted a position with the Bureau of Animal Industry as assistant coordinator of the cooperative beef cattle breeding program in the Southern region with headquarters at Knoxville, Tennessee.

Alvin C. Warnick, graduate of Utah Agricultural College and University of Wisconsin and an Air Force veteran, has been appointed physiologist in animal husbandry at the Oregon Agricultural College.

"Cattle Empire," by Lewis Nordyke, is the title of a new book on cattle development in the West, particularly Texas, published by William Morrow & Co., New York City.

U. S. Garrigus, who holds degrees from the University of Connecticut, and the University of Illinois, recently was made head of the sheep division of the Animal Science Department at the latter institution, and also secretary-treasurer of the Illinois Purebred Sheep Breeders' Association.

Ed. Hauser, who received training at the University of Wisconsin, Oklahoma A. & M., and the University of Missouri, and a Navy veteran with teaching experience at Clemson Agricultural College has been put in charge of beef cattle work at the University of Wisconsin.

Chas. R. Kyd of Missouri, now taking graduate work at Purdue University, has accepted the position of extension animal husbandman at Washington State College, to be effective July 1.

Ray R. Thalman, former animal husbandry staff member, University of Nebraska, has accepted a position with the American Hereford Journal as editorial and advertising representative on the West Coast.

James A. McGregor, animal husbandry graduate, University of Maryland, and World War II veteran, was appointed assistant extension animal husbandman of the Florida Extension Service, February 1, 1950.

Lewis F. Cato was appointed extension livestock specialist with the South Carolina Extension Service on January 1, and makes headquarters at Spartanburg in that state.

James K. Butler, Jr., recently was appointed part-time extension livestock specialist in the North Carolina Agricultural Extension Service with headquarters at Raleigh.

"Pigs from Cave to Cornbelt" is the title of a new book by Edward N. Wentworth and Charles Wayland Towne, published by the University of Oklahoma Press, Norman, Oklahoma.

E. J. Perry, Extension Specialist in Dairy Husbandry, College of Agriculture of Rutgers University, spent six weeks in Puerto Rico during February and March assisting the government in laying plans for setting up an artificial breeding program on the Island.

Donald A. Price has been appointed Animal Husbandman by the Bureau of Animal Industry, A.R.A., U. S. Department of Agriculture, and is stationed at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico.

Glen J. Spaulding has been appointed Animal Fiber Technologist in the Bureau of Animal Industry, Agricultural Research Administration, U.S.D.A., and is stationed at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico.

Paul B. Pearson, who joined the Atomic Energy Commission last March on leave of absence from Texas A. & M. College, has been appointed chief of the Division of Biology. In his new position, Dr. Pearson will be responsible for the biology program of the Commission. This involves work carried on in the National Laboratories, other AEC installations and research supported at colleges and universities.

E. F. Ferrin was promoted to the chiefship of the Division of Animal Husbandry, University of Minnesota, on December 12, as successor to W. H. Peters, deceased.

A. D. Weber, former president of the "Society," and head of the Animal Husbandry Department, Kansas State College, was advanced on January 1, 1950, to the rank of associate dean of the School of Agriculture, and associate director of the experiment stations at the same institution. Dr. Weber was succeeded by Rufus F. Cox as head of the Animal Husbandry Department.

Murl McDonald, assistant director of agricultural extension at Iowa State College since 1922, was recently confirmed by the State Board of Education as associate director of the same agency.

B. H. Schneider, professor of Animal Husbandry, West Virginia University, has resigned that position and became a member of the animal husbandry staff, Washington State College, on February 1.

Marvin B. Powell, who holds B.S. and M.S. degrees from Oklahoma A. & M. College, and recently head of the agricultural division of the Cameron State Agricultural College at Lawton, Oklahoma, was appointed extension specialist in animal husbandry at Kansas State College, effective September 1, 1949.

Edward Y. Hosaka, graduate of the University of Hawaii, was appointed assistant specialist in animal husbandry on the staff of the Hawaiian Extension Service, August 5, 1949.

Joseph Muir, extension animal husbandman, Washington State College, resigned that position to become county farm adviser in charge of livestock work, in Monterey County, California, effective January 1, 1950.

Dwight F. Stephens, with the Oklahoma A. & M. College since 1943, has been made superintendent of the El Reno beef cattle experiment station which is operated cooperatively by the U. S. Bureau of Animal Industry and the Oklahoma A. & M. College.

Paul G. Adams, formerly extension animal husbandman, Oklahoma A. & M. College, and more recently State 4-H Club leader, has accepted an assignment from the Office of Foreign Agricultural Relations, U.S.D.A., to serve as extension specialist in Nicaragua, C. A.

J. Matsushima, who recently received a Ph.D. degree from the University of Minnesota, is now assistant professor of Animal Husbandry at the University of Nebraska.

H. J. Smith, for some time member of the research staff at the Alabama Polytechnic Institute in animal breeding, has been appointed to a similar position at the University of Tennessee.

Ray G. Johnston, formerly of the animal husbandry staff, Oregon State College, and the recipient of foreign assignments to Asia and South America, has been appointed range management specialist with the Montana Extension Service.

James A. Christian, animal husbandry graduate from the Pennsylvania State College and Army Air Corps veteran, was recently named livestock specialist in the Extension Service at Penn State.

Reuben Albaugh, former assistant in Monterey County, California, has been made extension specialist in animal husbandry for the State Extension Service, with headquarters at Davis.

Joseph W. Houston, who holds degrees from Berea College and Texas A. & M. College, was appointed assistant animal husbandman in extension at the University of Tennessee, effective September 10, 1949.

Wilber H. Bruner, former county agricultural agent and more recently secretary of the Ohio Cooperative Wool Growers' Association, has been appointed specialist in livestock marketing in the Ohio Agricultural Extension Service.

Howard Davison, former extension swine specialist in the Ohio State Extension Service is now head of the swine department of the Producers' Livestock Cooperative Association at Columbus, Ohio.

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